BIOAVAILABILITY OF ORGANIC PHOSPHORUS IN A SHALLOW HYPEREUTROPHIC LAKE

By

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Abstract of Dissertation Presented to the Graduate School
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BIOAVAILABILITY OF ORGANIC PHOSPHORUS IN A SHALLOW HYPEREUTROPHIC LAKE

By

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Field and laboratory studies were conducted to determine the importance of organic P mineralization in the sediment-water column of Lake Apopka, a shallow hypereutrophic lake located in central Florida. Alkaline phosphatase activity (APA) was used as a tool to indicate the bioavailability of organic P to native plankton populations.

Spatial and temporal variability in total APA occurred in the water column (range=4 to 45 nM min^{-1}) in response to different water chemistry characteristics. Nutrient enrichment studies demonstrated that APA increased with plankton biomass and specific APA (APA/chlorophyll a) values > 1 nmol APA μg chlorophyll a^{-1} min^{-1} occurred during severe inorganic P limitation. In both the sediment and the water column APA was mainly associated with particulate matter.

The APA of the plankton was inhibited by high inorganic P concentrations. Phosphorus demand of the plankton was high, as evidenced by the rapid uptake of added inorganic P. During the conditions of P limitation added inorganic P was immediately assimilated and recovered in the surplus P pool within the plankton tissue, as determined by hot water extraction. The plankton apparently utilized this surplus pool of P for growth under low external inorganic P concentrations.

Resuspension of surficial sediments increased the interaction between sediments and the overlying water column, resulting in an immediate increase in APA and total P (TP) in the water column, indicating an increased potential for biological organic P hydrolysis during periods of resuspension. The APA and TP decreased rapidly during settling of suspended solids, following the cessation of turbulence.

Organic P mineralization was greater under aerobic than anaerobic conditions in the sediment and overlying water column. Under aerobic conditions (dissolved oxygen [DO]-6 mg L^{-1}) APA in the water column increased from 22 to 43 nM min^{-1}, while no change was observed under anaerobic conditions (DO=<0.2 mg L^{-1}). Sediment APA was a function of Eh, the measured reduction potential of the sediment-water systems. Under aerobic conditions (Eh=480 mV) APA was 10-fold higher than that observed under anaerobic conditions (Eh=-240 mV). Enzymatic hydrolysis of organic P compounds was significantly inhibited under anaerobic conditions.

The results from this study suggest that the P requirement of plankton in a highly productive lake may partially be met through the enzymatic hydrolysis of organic P. Consequently, efforts to reduce nutrient loading and thus reduce eutrophication should also evaluate the bioavailability of organic P compounds in the system.

CHAPTER 1
INTRODUCTION

Eutrophication may be defined as the nutrient and/or organic matter enrichment that produces high biological productivity (Likens 1972). This process is often accelerated by man, through allochthonous loading to the system from surface runoff, agricultural drainage and wastewater effluent.

Eutrophication of our waterbodies has recently become a major concern due to the ever increasing need for resource conservation. Consequently, efforts are now being made to further understand the process of eutrophication and to identify key management strategies to abate this process.

Statement of the Problem

Lake Apopka is a 12,500 ha lake located in central Florida. It has a mean water depth of 2 m, overlying highly flocculent organic sediments (Reddy and Graetz 1990). Historically, the lake had clear water, submersed macrophytes and supported substantial sport fish populations. However, the physico-chemical properties of the lake have been altered through nutrient enrichment following the construction of the Apopka-Beauclair canal, discharge of sewage to the lake, and back pumping from the surrounding muck farms (USEPA 1979). Following the 1947 hurricane, the submerged vegetation was uprooted, and the first algal bloom was recorded (USEPA 1979). Since then, sport fish populations have dwindled and have been replaced by rough fish such as shad, gar and catfish. The high algal populations have resulted in the maintenance of a pea-green color in the lake. Lake Apopka has mean chlorophyll a concentrations > 60 $\mu g\ L^{-1}$ and total P concentrations of 200 $\mu g\ L^{-1}$ (Canfield 1981; Huber et al. 1982; Reddy and Graetz 1990) and is thus currently classified as hypereutrophic (Forsberg and Ryding 1980). The lake is the first and largest in the Oklawaha chain of lakes, consequently a ripple effect is apparent. The high nutrient concentrations and algal blooms observed in Lake Apopka are evidenced downstream in the other lakes in the chain.

Need for Research

In order to understand the process of eutrophication in Lake Apopka and thus abate this process, it is necessary to determine the cycling of C, N and P in the sediment-water column. In general, N and P are the key elements involved in eutrophication (Chiaudani and Vighi 1982). Carbon fixation has been determined to be the driving force in the productivity of Lake Apopka (Reddy and Graetz 1990). This is apparent by the vast algal populations observed year round in the water column. Settling of senescent algal cells has resulted in a highly organic sediment. Consequently, both the sediment and the water column are dominated by organic matter which results in high levels of organic N and P. However, readily available P, i.e., soluble inorganic P concentrations are low and frequently undetectable (< 1 $\mu g\ L^{-1}$) (Newman, S., unpublished data, Department of Soil Science, University of

Florida, Gainesville, FL.). Researchers have investigated sorption reactions (Olila, O., unpublished data, Department of Soil Science, University of Florida, Gainesville, FL.) and have characterized the cycling of inorganic P within the sediment and water column (Pollman, 1983, Reddy and Graetz 1990), but the dynamics of the organic P pool, the dominant form of P, have not been addressed. Studies have demonstrated that significant quantities of organic P may be bioavailable (Bradford and Peters 1987, Kuenzler and Perras 1965), hence the organic P pool may play a significant role in sustaining the vast plankton biomass under apparent inorganic P limitation. Therefore, the potential bioavailability of organic P in Lake Apopka needs to be determined.

Organic Phosphorus Mineralization

In aquatic systems, organic P in sediments constitute 15-50% of TP (Boström et al. 1982) while in the water column organic P may account for as much as 90% of TP (Rigler 1964). The P cycle is shown in Fig. 1-1. Organic P is generally characterized as total and soluble organic P. Specific identification of organic P constituents may be achieved following chromatographic fractionation and comparison with known compounds (Minear 1972, Weimer and Armstrong 1979), ^{31}P nuclear magnetic resonance (NMR) (Condron et al. 1985), or via hydrolysis by specific enzymes (Herbes 1974). Only 50% of organic P forms have been identified, including inositol phosphates, sugar phosphates, phospholipids and nucleic acids (Stevenson 1992). The rate at which these compounds are mineralized is dependent upon their structure. High

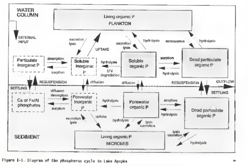

Figure 1-1. Diagram of the phosphorus cycle in Lake Apopka

molecular weight, complex structures are highly resistant to mineralization, hence they will tend to accumulate, e.g. humic acids. Conversely, simple, low molecular weight organic compounds are more susceptible to hydrolysis and thus more labile, e.g. sugarphosphates. It is these labile compounds which are more likely to undergo rapid enzymatic hydrolysis and thus be bioavailable.

Soluble reactive P (SRP), the most labile form of P, has been the focus of most P cycling research (Hutchinson and Bowen 1950, Rigler 1956). However, SRP concentrations in lakes are generally low while organic P is abundant (Abbott 1957, Rigler 1956). Such observations resulted in investigations to determine whether organic P compounds could act as a source of P available for plankton nutrition. Under inorganic P limiting conditions phytoplankton may utilize organic P compounds for growth (Harvey 1953, Kuenzler 1965). These phytoplankton produce externally acting enzymes, phosphatases which hydrolyze phosphomonoesters (PME) and release SRP (Fitzgerald and Nelson 1966, Kuenzler and Perras 1965). Phytoplankton which do not producce externally acting enzymes cannot hydrolyze PME compounds and their growth becomes P limited (Kuenzler 1965). Ecologically, the ability of phytoplankton to utilize organic P gives them a competitive advantage over non-phosphatase producers, during inorganic P limitation.

The mode of action of phosphatases (specifically phosphomonoesterases) is shown below (Coleman and Gettins 1983).

$$ROP + E \overset{1}{\rightleftharpoons} ROP\,E \overset{2}{\rightleftharpoons} E\text{-}P \overset{3}{\rightleftharpoons} E\cdot P \overset{4}{\rightleftharpoons} E + P_i$$

R = an organic moiety, P_i = inorganic P, E = enzyme.

The steps involved in the reaction are as follows:

1 The phosphomonoester binds non-covalently to the phosphatase enzyme (ROP•E).

2. The phosphoseryl intermediate forms by covalent binding of the phosphate group to the phosphatase enzyme (E-P); alcohol is released during this nucleophilic attack.

3. Water is taken up resulting in the nucleophilic displacement of serylphosphate to produce a non-covalently bound complex (E•P).

4 Inorganic P is released and the free phosphatase enzyme is regenerated.

Phosphatases have a high degree of specificity for the P moiety of the P-O-C bond, but little specificity for the C moiety (Reid and Wilson 1971). These enzymes are classified as alkaline or acid depending on the pH range under which they exhibit optimum activity (Reichardt 1971; Torriani 1960). At acid pH, the dephosphorylation of the serylphosphate is the rate limiting step. At alkaline pH, the dissociation of inorganic P from E•P is the rate limiting step (Coleman and Gettins 1983). The alkaline nature of most aquatic systems has resulted in alkaline phosphatase activity (APA) receiving the most emphasis.

More than one type of phosphatase may be present in any plankton population. Five intracellular phosphatases were extracted from a *Peridinium* bloom (Wynne 1977). The phosphatases produced in response to P limitation do not have the same biochemical characteristics as those observed in normal tissues (Bielski 1974). Although acid phosphatases have the ability to function outside the cell (Kuenzler and Perras 1965;

Price 1962), they are generally intracellular in action (Moller et al 1975, Wynne 1977). Acid phosphatases function as specific enzymes in metabolic pathways and non-specific reactions (Cembella et al 1984a). Hence they are constitutive and generally not repressible by inorganic P. Conversely, APA exhibits principly extracellular function. Alkaline phosphatase synthesis may be induced by the presence of organic P (Aaronson and Patni 1976, Kuenzler 1965). Alkaline phosphatase is a repressible enzyme (Jansson et al. 1988), whose synthesis is inhibited by high levels of inorganic P (Elser and Kimmel 1986, Lien and Knutsen 1973, Torriani 1960). Inorganic P is a competitive inhibitor of APA (Coleman and Gettins 1983, Moore 1969, Reid and Wilson 1971). Other factors which affect APA include, temperature (Garen and Levinthal 1960; Torriani 1960), chelators and divalent cations (Cembella et al. 1984a, Healey 1973).

The derepression of APA in response to P limitation has been examined at the cellular level where APA was shown to transport inorganic P. Studies utilizing *Escherichia coli* have shown that two forms of P transport exist (Rosenberg et al. 1977). One is a low affinity system, phosphate inorganic transport (PIT), which is constitutive and transfers intracellular P pools. The other is a high affinity system, phosphate specific transport (PST), which is activated when internal P concentrations are low. This utilizes a membrane associated protein, APA, to increase P uptake. The high affinity system is inhibited at high concentrations of inorganic P. However, it is the ability of APA to catalyze the hydrolysis of organic P compounds that has received the most study in the aquatic environment.

The intensity of APA is dependent on the severity of P limitation. As much as 6% of the total protein produced under P limiting conditions may be attributed to APA (Garen and Levinthal 1960). The increase of APA in response to inorganic P deficiency has resulted in the use of APA as a tool to assess the P limitation of plankton.

Alkaline Phosphatase Activity in the Water Column

Inverse relationships between APA and SRP have been reported in many species of plankton (Healey 1973, Olsson 1990, Pettersson 1980, Pettersson et al. 1990). Under low SRP concentrations APA is derepressed, and upon replenishment of external inorganic P, APA is inhibited. In some situations no significant relationship is observed between APA and SRP (Berman 1970; Taft et al. 1977). It has been suggested that under these circumstances high concentrations of soluble organic P counteract the inhibition caused by SRP by stimulating induction of APA (Kuenzler 1965, Cembella et al. 1984a). Alternatively, where no correlation exists, APA may reflect P demand rather than P limitation (Taft et al. 1977).

In combination with the depletion of external concentrations of inorganic P, P limitation in plankton is also demonstrated by reduced internal P concentrations (Chróst and Overbeck 1987, Rhee 1973). Inverse relationships between APA and surplus P have been recorded (Lien and Knutsen 1973, Rhee 1973). Once internal P concentrations have been reduced below critical levels, APA is produced (Chróst and Overbeck 1987, Fuhs et al. 1972). Alkaline phosphatase activities have been observed to be 25 times greater under P limitation than under P sufficiency (Fitzgerald and Nelson 1966).

Ecologically, the importance of APA is dependent on the co-occurrence of both substrates and enzymes. Numerous problems have been associated with the determination of PME concentrations. The most common method of determining PME has been to monitor SRP release from filtered lake water following the addition of pure alkaline phosphatase (Strickland and Parsons 1968). The simultaneous occurrence of PME and APA by cyanobacteria blooms has been observed under low SRP concentrations (Heath and Cooke 1975). In some lakes, the rate of inorganic P release from PME equals the rate of P uptake by the plankton. In other lakes a large discrepancy exists between these two rates, with inorganic P release being considerably less than uptake rate (Boavida and Heath 1988; Cotner and Heath 1988; Heath 1986), thus leading these researchers to conclude that APA is not important in P nutrition of plankton. One of the problems associated with this conclusion is the use of filtered lake water in the analyses, therefore the large particulate organic P pool is absent (Wetzel 1983). Phosphatases have also been shown to release P from particulate matter (Jansson 1977). Seventy four percent of extractable P in phytoplankton is susceptible to enzymatic hydrolysis and 80% of the organisms involved in phytoplankton decomposition produce phosphatases (Halemejko and Chróst 1984). These results, and the apparent absence of hydrolyzable soluble PME in the water column (Herbes 1974, Herbes et al. 1975, Pettersson 1980) suggest that it is the substrate availability that limits enzymatic P cycling not APA (Jansson et al. 1988).

The interpretation of APA as a measure of P limitation is complicated by the uncertainty of the origin of APA. Bacteria, phytoplankton and zooplankton are considered to be dominant contributors to this pool, and it is suggested that APA of algal origin is the most important in the epilimnion (Jansson et al. 1988). High levels of soluble APA indicate filterable activity and may reflect bacterial associated APA (Stewart and Wetzel 1982), zooplankton excretion (Jansson 1976; Wynne and Gophen 1981) and cell lysis (Berman 1970). In many lakes APA was determined to be mainly associated with phytoplankton, based on co-occurrence of APA and algal blooms (Heath and Cooke 1975), and as shown by correlations with chlorophyll a (Jones 1972a, Matavulj and Flint 1987, Siuda et al. 1982, Smith and Kalff 1981) and size fractionation of phosphatase activity (Chróst et al. 1989, Jansson 1977). Alkaline phosphatase activity has also been attributed to bacteria through correlations with bacterial numbers (Jones 1972a; Kobori and Taga 1979a). In shallow lakes, a large portion of particulate material may be sedimentary in origin. Concentrations of P compounds and bacterial numbers may be higher in sediments. Interaction between sediment and the overlying water column may significantly affect the mineralization of organic P in the overlying water. Hence, wind events in shallow lakes can significantly affect APA.

Alkaline Phosphatase Activity in the Sediment

In lake sediments as much as 70% of TP can be in organic form (Weimer and Armstrong 1979). In highly organic sediments, the relative abundance of organic substrates may result in enhanced breakdown of organic P (Ayyakannu and Chandramohen 1971). Numerous enzymes can be utilized in organic P breakdown but the phosphatases, specifically APA, are the most frequently cited (Halstead and McKercher 1975, Skujins 1978, Speir and Ross 1978).

As observed in the water column, APA in soils is positively correlated with the concentration of organic matter (Harrison 1983, Speir 1978) and age of organic matter (Rojo et al. 1990). Much of the data concerning APA have been developed in upland soils (Geller and Dobrotvorskaya 1981, Juma and Tabatabai 1978, Tabatabai and Bremner 1969), few studies have investigated APA in sediments. However, highly significant APA has been reported in both freshwater (Klotz 1985a, Sayler et al. 1979) and marine sediments (Ayyakannu and Chandramohen 1971, Kobori and Taga 1979b).

Phosphatase activity decreases with soil (Juma and Tabatabai 1978) and sediment depth (Degobbis et al. 1984, Kobori and Taga 1979b). In upland soils, this has been shown to correspond to decreases in microbial biomass, C, N and organic P (Juma and Tabatabai 1978, Speir and Ross 1978, Baligar et al. 1988). In sediments, redox potential decreases significantly with depth, therefore an important distinction between mineralization of organic compounds in sediments versus the overlying water column is the concentration of oxygen. Limited oxygen diffusion and rapid consumption of oxygen results in an oxygenated layer at the sediment-water interface and decreasing oxygen with depth in the sediment (Charlton 1980, Boström et al. 1982). Alkaline phosphatase activity is generally inhibited under anaerobic conditions, resulting in a slower rate of organic P mineralization (Pulford and Tabatabai 1988).

However, in shallow lakes, wind induced resuspension of sediments to the oxygenated water column, results in the rapid breakdown of organic P (Pomeroy et al. 1965). A significant positive correlation between SRP released and APA in the water column has been observed during sediment resuspension (Degobbis et al. 1984).

Resuspension can physically transport SRP to the overlying water column (Ryding and Forsberg 1977). It also increases the suspended solids concentration within the overlying water. This particulate material can provide 28-41% of algal available P (Dorich et al. 1985). Consequently, resuspension of sediments can result in enhanced enzyme activity and P availability within the water column. Hence sediments can play a significant role in organic P mineralization in the overlying water column.

Objectives

Bioavailability of organic P occurs through the action of enzymes (Kuenzler and Perras 1965). These enzymes catalyze the release of inorganic P from both soluble and particulate matter. Both APA and organic P concentrations increase with increased eutrophication (James 1979b). Hence, Lake Apopka, which is classified hypereutrophic, should support large concentrations of APA and organic P. This study is based on the hypothesis that enzyme mediated P release is used to support the vast algal populations during inorganic P limitation. Without this ability to utilize organic P at times of high P demand and inorganic P limitation, algal and bacterial species are nutrient stressed. Very little is known about the bioavailability of organic P in sub-tropical lakes, consequently, research investigating the breakdown and utilization of organic P is essential in any assessment of lake eutrophication.

The main components influencing the cycling of organic P within the water column are, water chemistry, plankton uptake and release, and sediment resuspension (Fig 1-1). To understand the role of organic P in Lake Apopka the following questions were addressed.

(1) How is the enzymatic hydrolysis of organic P compounds affected by other water chemistry parameters?

(2) Is Lake Apopka plankton APA inhibited by inorganic P and is it produced in response to inorganic P limitation?

(3) What effect does sediment resuspension have upon organic P mineralization rates?

The overall objective of this study was to evaluate the significance of organic P compounds in Lake Apopka and determine their potential bioavailability. Specific objectives are listed below.

(1) Determine the seasonal, spatial and diel variability of APA within the water column.

Alkaline phosphatase activity is produced by organisms, hence any factors such as changes in environmental conditions which affect metabolism may therefore affect APA. The predominant biotic group in Lake Apopka biota are the plankton, hence APA is linked to fluctuations in response to plankton metabolism.

(2) Determine the influence of inorganic P upon the growth of natural plankton populations.

Soluble inorganic P is the most readily available form of P for plankton nutrition, however, in its absence organic P compounds may be used for growth. The enzymatic hydrolysis of organic P compounds is competitively inhibited by inorganic P. Soluble reactive P concentrations in Lake Apopka are hypothesized to be too low to inhibit APA. There is, however, the issue of internal concentrations of P which may regulate organic P hydrolysis outside the cell.

(3) Evaluate the effect of sediment resuspension upon the mineralization of organic P in the sediment and overlying water column.

In shallow lakes, wind induced resuspension of sediments into the overlying water column increases the interaction between these compartments. It is hypothesized that resuspension of sediment increases the concentration of organic substrates and associated microorganisms in the water column and thus increases mineralization.

(4) Determine the effect of anoxia on organic P mineralization in the sediment and water column.

Mineralization of organic compounds proceeds more rapidly under aerobic than anaerobic conditions. Since a majority of sediments are anaerobic, it is hypothesized that APA will be inhibited under anaerobic conditions, resulting in a reduced mineralization rate.

Dissertation Format

Each chapter within this dissertation is written as an independent manuscript intended for future publication. Chapter 2 focuses upon the concentrations of P compounds and APA within the water column and the various factors which affect them. Chapter 3 examines the P nutrient status of natural plankton populations. Chapter 4 investigates the effect of sediment water column interactions upon organic P bioavailability. The effect of anaerobic/aerobic conditions on organic P bioavailability is examined in chapter 5. The overall conclusions and the significance of these results are discussed in chapter 6.

CHAPTER 2

SEASONAL VARIABILITY IN ALKALINE PHOSPHATASE ACTIVITY IN A SHALLOW HYPEREUTROPHIC LAKE

Introduction

Phosphorus is the major nutrient limiting plankton production in many temperate lakes (Schindler 1977). Soluble reactive P (SRP) has been the form of P most often studied (Rigler 1956); however, SRP is only a small fraction of the total P (TP) pool. A significant component of TP may be in organic form (Minear 1972, Rigler 1964). In lakes where inorganic P availability is low, plankton may produce phosphatase enzymes which hydrolyze organic P compounds with the release of inorganic P (Fitzgerald and Nelson 1966, Kuenzler and Perras 1965). These enzymes are designated alkaline or acid phosphatase, depending on the pH range of optimum activity (Kuenzler and Perras 1965, Torriani 1960). The alkaline nature of most water bodies has resulted in alkaline phosphatase activity (APA) receiving the most attention. Although some APA has been determined to be constitutive (Kuenzler 1965), plankton produce increased APA under conditions of P limitation. Alkaline phosphatase activity has hence been used as an indicator of P limitation, however, APA may also reflect P demand, as evidenced by a poor relationship between SRP and APA (Taft et al. 1977).

The release of inorganic P mediated by APA is dependent upon the percentage of organic P which is hydrolyzable by the enzyme. Thirty-six percent of organic P in seawater (Kobori and Taga 1979a) and 32% of organic P in freshwater (Hino 1988) were hydrolyzed by phosphatase enzymes. Organic P of algal origin is particularly sensitive to hydrolysis; 74% of algal extracted P was hydrolyzed by APA, while 80% of organisms involved in the decomposition of plankton produced phosphatases (Halemejko and Chróst 1984). In eutrophic situations TP and organic P concentrations can be high (Jones 1979b), resulting in higher APA levels than in lower trophic states (Jones 1979b, Pick 1987). Alkaline phosphatase activity may be a significant mechanism of satisfying high P demand in eutrophic situations, as well as a means of overcoming P limitation in nutrient poor environments. The intensity of APA is subject to the physico-chemical conditions in the environment. Enzyme activity is pH dependent and can respond negatively or positively to pH fluctuations (Torriani 1960). Dissolved oxygen (DO) and temperature also influence microbial enzyme activity and metabolism, thus they may directly or indirectly affect APA (Garen and Levinthal 1960). These environmental effects demonstrate the potential for seasonal and diel fluctuations in APA.

This chapter examines the impact of seasonality on APA in one of Florida's largest hypereutrophic lakes, Lake Apopka. Despite low SRP concentrations < 1 $\mu g\ L^{-1}$, chlorophyll *a* concentrations are regularly > 100 $\mu g\ L^{-1}$ (Canfield 1981, Reddy and Graetz 1990). High standing crops may be maintained through the rapid recycling of SRP or by obtaining P from other sources. Total soluble P (TSP) concentrations of 255 μg P L^{-1} have been recorded in Lake Apopka (Reddy and Graetz 1990). Total soluble P may represent bioavailable P (Bradford and

Peters 1987), hence organic P compounds in this pool may potentially be hydrolyzed by APA and release inorganic P. With the exception of extensive research by Berman and colleagues on Lake Kinneret, Israel (Berman 1970; Wynne and Berman 1980), the majority of studies investigating APA have been conducted in cold temperate zones. Warmer climates with mild winters which result in extended periods of productivity, may result in increased P demand. More studies in warmer climates are necessary.

The primary objective of this study was to examine the seasonal, spatial and diel changes in APA to determine whether it represents P limitation or high P demand. This would also determine what effect the water chemistry has upon APA. Zooplankton, phytoplankton and bacterioplankton may all contribute to the total APA pool (Jansson 1976; Jones 1979a, Kuenzler and Perras 1965, Wynne and Gophen 1981). A second objective was to estimate the relative importance of these contributors based on filter size fractionation. Total soluble P may be used as an indicator of bioavailable P, however, a third objective was to determine the relationship between APA and other components of the TP pool.

Materials and Methods

Site Description

Lake Apopka is a 12,500 ha, located in central Florida, 28° 37′ N latitude, 81° 37′ W longitude (Fig. 2-1). It has a mean depth of 2 m. Water influxes to the lake include Apopka Springs and backpumping from surrounding agricultural land. Outflow is northward through the Apopka-

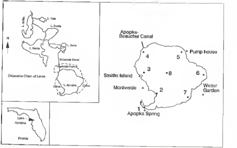

Fig. 2-1. Location of Lake Apopka and sampling sites

Beauclair canal. The St. John's River Water Management District (SJRWMD) weather station is located at the center of the lake (site 9).

Water Sampling

Bimonthly sampling. Lake water was collected bimonthly from April 1989 thru February 1990, from 9 sites in Lake Apopka (Fig. 2-1). Sites 1, 4 and 9 were selected to represent inflow, outflow and the center of the lake. Site 2 was selected to determine littoral zone influences. Site 5 was located close to a pump station and hence represented backpumping from the surrounding agricultural land. Site 7 was established close to an old fishing camp, and site 6 was selected to correspond to extensive sediment studies which were conducted with samples from that site. Site locations were established using Loran coordinates (Appendix A). Three replicate water samples were collected from a depth of 0.3 m using 1 L polyethylene bottles, from each site. Samples were stored on ice until return to the laboratory. Water was filtered through 0.45 μm membrane filters (Gelman) and analyzed for soluble APA within 24 h. Other soluble parameters determined were total soluble P and SRP. Soluble particulate P was defined as TSP-SRP (SPP). Whole lake water was analyzed for total APA, total Kjeldahl N (TKN), TP, SRP, total solids (TS), total suspended solids (TSS), total organic carbon (TOC), and chlorophyll *a*. Seasonal water chemistry data collected at site 9 were compared with the weather data provided by the SJRWMD.

The contributors to the APA pool are frequently determined via filter fractionation (Chróst and Overbeck 1987, Currie and Kalff 1984, Currie et al. 1986). To distinguish between the contribution of phytoplankton and bacteria, water samples collected from sites 1, 4 and 8 received further filtration. Subsamples of water were filtered through 150, 8, 2.5, 0.45 and 0.2 µm filters. Water from these size fractions was analyzed for chlorophyll *a* and determined to represent 80, 9, 3, 1 and 0% chlorophyll *a* distribution. To minimize the filtration time, the filtration was not performed sequentially. Alkaline phosphatase activity and TP were determined on all samples.

Dissolved oxygen and temperature (YSI, model 58), and pH (Orion, model SA 230) were recorded at 0.3 m. Light penetration was estimated by measuring Secchi disk transparency. Samples were collected at approximately the same time at each sampling period to minimize the effects of diel variation.

Diel sampling. Diel studies were conducted on March 21 1989 and February 6 1990. In March 1989 DO and pH of the water were measured from a pontoon boat which was anchored at the central station (site 8) for 24 h. In February 1990, pH and DO measurements were determined by SJRWMD personnel. Water samples at both time periods were collected using an automatic sampler, and kept cool until returned to the laboratory for analysis.

Fractionation of lake water phosphorus. To elucidate the relationship between P and APA in the water column, the P forms were separated analytically using discrete extraction procedures. In October 1989, water samples from all 8 sites were partitioned into total and soluble, reactive P, acid hydrolyzable and organic P (APHA 1985). Enzyme hydrolyzable P (EHP) was also determined.

Analytical Methods

Alkaline phosphatase activity was determined fluorometrically (Healey and Hendzel 1979). One half mL of substrate, 3-o-methylfluorescein phosphate (Sigma Chemicals), at a concentration determined to be non-limiting, in 0.1 M Tris buffer (Tham, Fisher Scientific) pH 8.6, was added to 4.0 mL of lake water in a cuvette. Both total (whole lake water) and soluble (filtered through 0.45 µm Gelman membrane filter) APA were determined. The cuvettes were placed in a water bath (25°C). At timed intervals during a 20 min period the cuvettes were placed in the fluorometer and the fluorescence measured. The enzyme activity was measured as an increase in fluorescence as the substrate was enzymatically hydrolyzed to the fluorescent product. Fluorescence units were converted to enzyme activity using a standard calibration curve of 3-o-methylfluorescein (Sigma Chemicals). The fluorescence was measured using a Turner fluorometer No. 110, equipped with Turner lamp no. 110-853, in combination with 47 B primary and 2a-12 secondary filters. Autoclaved lake water with substrate added was used as a control.

Chlorophyll *a* was determined spectrophotometrically following extraction with acetone (APHA (1002-G), 1985). Total organic C was measured using an O. I. Corporation Model 524C TOC analyzer, following oxidation by potassium persulfate. Total P, TSP, and TKN were determined following Kjeldahl digestion. Soluble reactive P, TSP and TP were analyzed via ascorbic acid using standard methods (APHA 1985). Total solids and TSS were determined by standard methods (APHA 1985).

Enzyme hydrolyzable P was determined using the method of Strickland and Parsons (1968).

Statistics

Data were analyzed using SAS for personal computers, version 6 (SAS 1985). Pearson correlation coefficients were determined for all data.

Results

Measured parameters varied seasonally and spatially. Seasonal variability was generally greater than spatial variability (data presented graphically represent means of sites 2-9). Appendix B contains tables presenting data on a site basis and demonstrates spatial variability. Site 1 is the site of a natural spring, 90 ft deep and is not subject to the same wind induced mixing as the rest of the lake. Data from site 1 are discussed separately, to illustrate the effects of spring input to the lake.

Physico-chemical Characteristics

A 14°C range in water temperature was observed over the sampling period (Table 2-1). The highest water temperatures occurred in June and August, and coincided with maximum photosynthetically active radiation (PAR) (Table 2-1). Temperatures were coolest in October and December during the decline of daylength. Data for other physical and chemical characteristics are presented in Appendix B. Increased Secchi transparency was recorded during October and December. Dissolved oxygen concentrations ranged from 7 to 12 mg L^{-1}, with a mean of 9.9 mg L^{-1}.

Table 2-1. Means of selected weather data measured at the central station (mean ± 1 SE).

Date	Wind speed 1 m above surface	 5 m	Water temp	PAR[*]
ym	---- km h^{-1}	----	°C	µmol m^{-2} s^{-1}
8904	2 ± 0	NA[•]	23.6	NA
8906	9 ± 0	10 ± 0	29.8	548 ± 29
8908	11 ± 0	13 ± 0	28.5	508 ± 29
8910	11 ± 0	20 ± 0	19.4	238 ± 14
8912	11 ± 0	13 ± 0	15.5	294 ± 18
9002	13 ± 0	18 ± 0	20.6	282 ± 18

[*] PAR indicates photosynthetically active radiation.

[•] NA indicates data not available.

Source. Stites, D. L., unpublished data, St. John's River Water Management District, Palatka, FL.

Little variation in pH was observed with a range of 0.9 pH units observed between maximum and minimum pH. Total solids were also constant between 300 and 400 mg L^{-1}, until February when a particularly high concentration of 500 mg L^{-1} was recorded. Total suspended solids accounted for approximately 16% of TS and increased in February at all sites (Fig. 2-2a).

A distinct peak in chlorophyll a concentration was observed in August at 5 of the 7 sites (Fig. 2-2b). This peak was 3 fold higher than the minimum which occurred in February. Two peaks in TOC were apparent. One occurred in August, along with chlorophyll a (Fig. 2-2c) while the other occurred in February and probably corresponded to the increase in TSS which also occurred in February. Total Kjeldahl N tended to be higher in Spring and Summer and decreased in Fall and Winter (Fig. 2-2d).

Alkaline phosphatase activity was mainly associated with particulate matter (Fig. 2-3a). Soluble APA averaged only 3% of total APA. Total APA peaked in both June and October, in contrast, soluble APA peaked in October and December.

Phosphorus was also determined to be mainly associated with particulate matter. Total P concentrations peaked in October (Fig. 2-3b). Total soluble P concentrations represented from 6 to 37% of TP in August and April respectively. Soluble reactive P concentrations were very low throughout the year (0 to 7 µg L^{-1}) and as such were a minor portion of TP.

All parameters measured at site 1 were generally much lower than those recorded at other stations (Appendix 9). In particular DO and pH

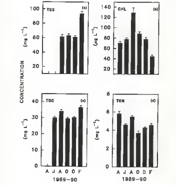

Fig. 2-2. Seasonal variability of selected parameters determined bimonthly at 7 sites in Lake Apopka. a) total suspended solids, data were not collected in April and June, b) chlorophyll *a*, c) total organic carbon, data were not collected in April, d) total Kjeldahl nitrogen. Vertical bars represent 1 SE.

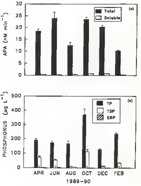

Fig. 2-3. Seasonal variability of selected parameters determined bimonthly at 7 sites in Lake Apopka a) alkaline phosphatase activity, b) phosphorus. Vertical bars represent 1 SE.

were considerably lower and Secchi was significantly greater. Apopka spring temperatures tend to be consistent throughout the year, only a 4°C fluctuation in water temperature was observed. Algal biomass was significantly lower than observed in the rest of the lake. Annual chlorophyll a concentrations averaged 21.0 $\mu g\ L^{-1}$ at site 1, while values averaged 81 $\mu g\ L^{-1}$ at other sites.

The contribution of the various components in lake water to the total APA pool was determined via filter size fractionation. The distribution of APA followed that of chlorophyll a, with the majority of the activity associated with the larger size fraction (Fig. 2-4), and the distribution of APA within the different size fractions remained constant throughout the year. The greatest amount of soluble APA occurred in December. In general, a greater proportion of APA was associated with 8 and 2.5 µm filtered samples in spring water at site 1, than was observed in samples from sites 4 and 9. A similar distribution was also determined for P (Fig. 2-5), although a greater proportion was associated with the soluble fraction. Phosphorus concentrations peaked in October at all three sites.

Relationships between Water Chemistry Data and Selected Environmental Parameters

Seasonal patterns in PAR, wind speed and water temperature collected at site 9 (lake center) were observed (Table 2-1). Water temperature and PAR peaked in June and August. Wind speed measured 5 m above the surface was generally greater than that measured 1 m above the surface (Table 2-1). Total P was positively correlated with wind speed observed 5 m above the water surface (r=0.88) and SRP was

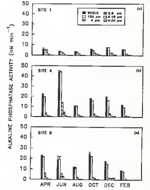

Fig. 2-4. Size fractionation of alkaline phosphatase activity determined bimonthly at 3 sites in Lake Apopka. a) site 1=inflow, b)site 4=ouflow, c) site 8=center of lake. Vertical bars represent 1 SE

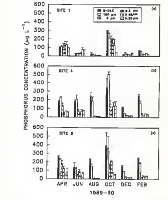

Fig 2-5. Size fractionation of phosphorus concentrations determined bimonthly at 3 sites in Lake Apopka. a) site 1=inflow, b)site 4=ouflow, c) site 8=center of lake. Vertical bars represent 1 SE.

positively correlated with PAR (r=0.99). Total Kjeldahl N was inversely related to wind speed measured 1 m above the surface (r=-0.94), while TSS was highly positively correlated with the wind speed recorded 1 m above the surface (r=0.97). Total suspended solids were also positively correlated with chlorophyll *a*, and inversely correlated with TSP and SOP. Neither chlorophyll *a* nor APA were correlated with any of the weather data.

Relationships between Alkaline Phosphatase Activity, Chlorophyll *a* and other Parameters

Alkaline phosphatase activity did not correlate with many water chemistry parameters (Table 2-2). A significant correlation was observed between chlorophyll *a* and total APA in December (r=0.82), while APA was inversely related to TSP (r=-0.86). Correlations among chlorophyll *a*, total APA and other measured parameters varied over time. Chlorophyll *a* correlated with P four out of the six sampling periods and total APA only correlated with P twice (Table 2-2). On a site by site basis, different correlations between APA and water chemistry parameters were observed (Table 2-3). Spatial variability of the factors affecting APA was apparent.

Utilizing annual means, total APA was negatively correlated with TOC (r=-0.97) and chlorophyll *a* was highly correlated with TS (r=-0.88). Specific APA (ratio of total APA/chlorophyll *a*) was not correlated with any of the water chemistry parameters.

Diel Variability

On March 21 1989 and February 6 1990 selected parameters influencing APA were measured over a 24 h period to determine diel

Table 2-2. Correlation coefficients for chlorophyll *a* and alkaline phosphatase activity measured bimonthly at 7 sites in Lake Apopka (significant at α=0.05, n=7)

Month	Correlations with		
	Chlorophyll *a*	Total APA	Soluble APA
April	TKN 0.88 TP 0.85 DO 0.74 temp -0.96	TP 0.75 SPP -0.67	NS*
June	TKN 0.92 TSP 0.76 TOC 0.69 SPP 0.78	soluble APA 0.74 DO -0.76	pH 0.79
August	NS	NS	NS
October	TOC -0.70 TSS 0.73 SPP -0.66	NS	TKN -0.75 TP 0.75 temp 0.71 TS 0.67
December	total APA 0.82 temp 0.68 secchi 0.80	TSP -0.86 SPP 0.86	TP 0.70 TSP 0.81 SPP 0.81
February	TP 0.71	TOC -0.69	NS

* NS indicates not significant at α = 0.05.

Table 2-3. Correlation coefficients between alkaline phosphatase activity and parameters measured bimonthly at 8 sites in Lake Apepka (significant at α=0.05, n=6)

Site	Alkaline phosphatase activity			
	Total		Soluble	
1	DO	0.87		
	temp	-0.81	temp	-0.77
2	TKN	-0.78	TKN	-0.90
	TOC	0.81	TP	0.89
			TSP	0.85
			SPP	0.86
			secchi	0.85
3	soluble APA	0.82	TP	0.75
	secchi	0.79	secchi	0.79
4	NS*		CHL	0.75
			DO	0.92
5	TP	0.75	secchi	0.90
6	TSS	-0.93	secchi	0.81
7	NS		TP	-0.83
			pH	0.73
8	TSP	0.83	NS	
	SPP	0.81		
	pH	0.80		
	DO	0.82		
	TOC	-0.92		

* NS indicates not significant at α = 0.05

variation. Diel DO changes were observed at both time periods. No significant changes in other measured water chemistry parameters were observed in March 1989, while parameters did exhibit change in February 1990. In February 1990, TKN concentrations remained constant during the first 12 h of sampling and then declined (Fig. 2-6a). Maximum TKN corresponded to high PAR and wind speed (Fig. 2-7a and 2-7b). The decline in TKN corresponded to the decrease of these two parameters. No significant change was observed for SRP, while TP concentrations fluctuated throughout the sampling period (Fig. 2-6b). Similar fluctuations were also observed for total and soluble APA (Fig. 2-8a). As observed for TKN, chlorophyll a concentrations tended to decrease in conjunction with decreased PAR and wind speed, however, the range was only 33 to 37 $\mu g\ L^{-1}$ (Fig. 2-8b).

Fractionation of Lake Water Phosphorus

In October, lake water samples were fractionated to determine the different forms of P present. Site variability in chlorophyll a and TSS concentrations was observed (Appendix B). Total organic C remained constant at 30 $mg\ L^{-1}$ for all sites except site 1. Chlorophyll a and TOC were lower at site 1.

As determined above, most of the total APA was in the particulate fraction with the soluble APA contribution varying from site to site (Appendix B). The distribution of the various P forms also exhibited spatial variability (Fig. 2-9). In most sites TOP represented over 80% of TP. Total acid hydrolyzable P contributed 10% and total reactive P (TRP) contributed 3% to the TP pool. A similar distribution of these

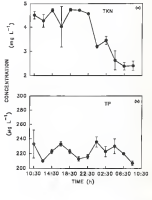

Fig 2-6. Diel variation of selected paramters measured February 6-7, 1990 at the center of Lake Apopka (site 8) a) total Kjeldahl nitrogen, b) total phosphorus. Vertical bars represent 1 SE.

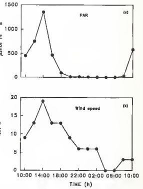

Fig. 2-7. Diel variation of selected parameters measured February 6-7, 1990 at the center of Lake Apopka (site 8) a) photosynthetically active radiation, b) wind speed 1 m above the water surface. Source. Stites, D. L., unpublished data, St. John's River Water Management District, Palatka, FL.

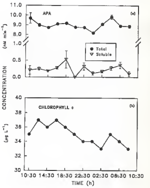

Fig. 2-8. Diel variation of selected parameters measured February 6-7, 1990 at the center of Lake Apopka (site 8). a) alkaline phosphatase activity, b) chlorophyll *a*. Vertical bars represent 1 SE. Chlorophyll *a* values were measured on composite samples.

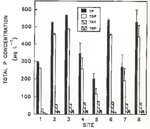

Fig. 2-9. Distribution of phosphorus compounds determined in whole lake water at 8 sites in October 1988. TP=total phosphorus, TOP=total organic phosphorus, TAH=total acid hydrolyzable phosphorus, TRP=total reactive phosphorus. Vertical bars represent 1 SE.

components was also observed in filtered lake water (Fig. 2-10), organic P represented > 90% of TSP. Soluble acid hydrolyzable P was a less significant contributor to the total soluble P pool. Soluble reactive P concentrations were negligible. Total soluble P accounted for 11 to 60% of TP at sites 9 and 7, respectively. The fraction of TP attributed to suspended material was determined by difference between total and soluble P fractions (Fig. 2-11). The distribution of P forms was similar to that observed in whole lake water. Suspended TP represented from 40 to 59% of TP. No enzyme hydrolyzable P was observed.

Correlating all the site means (including site 1), total APA was positively correlated with chlorophyll *a* (r=0.88), TOC (r=0.84) and TSS (r=0.85). However, plots of the data showed that these correlations were an artifact of low values for water chemistry parameters at site 1. Correlations without site 1 gave different conclusions (Table 2-4). Total APA was observed to be inversely correlated with the acid hydrolyzable fractions. Chlorophyll *a* was inversely correlated with TOC (r=-0.68) and TSP (r=-0.66) and positively with TSS (r=0.73).

Discussion

Lake Apopka is a shallow lake with a surface area of 12,500 ha. It has a small littoral zone and is subject to considerable wind induced sediment resuspension. Frequent mixing and mild winters may help to explain the lack of seasonality observed for several of the parameters measured. Chlorophyll *a*, however, did exhibit a seasonal response. Maximum chlorophyll *a* corresponded to high PAR and higher temperatures. Over all months, chlorophyll *a* was only significantly and

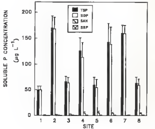

Fig 2-10 Distribution of phosphorus compounds determined in filtered lake water at 8 sites in October 1989. TSP=total soluble phosphorus, SOP=soluble organic phosphorus, SAH=soluble acid hydrolyzable phosphorus, SRP=soluble reactive phosphorus Vertical bars represent 1 SE.

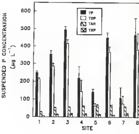

Fig. 2.11. Distribution of suspended phosphorus compounds determined by difference between whole and soluble P measured at 8 sites in October 1989. TP=total phosphorus, TOP=total organic phosphorus, TAH=total acid hydrolyzable phosphorus, TRP=total reactive phosphorus. Vertical bars represent ± SE.

Table 2-4 Correlation coefficients of selected water chemistry parameters determined at 7 sites in October 1989 (significant at α=0.05, n=7)

	TP	TRP	TAH	TOP	TSP	SAH	SOP	TSUSP	SUSAHP	CHL
TOP	1.00*									
SAH			0.92							
SOP					1.00					
TSUSP	0.95			0.94						
SUSRP		0.79								
SUSAHP			0.99			0.86				
SUSOP	0.97			0.86				0.99		
TAPA			-0.80			-0.73			-0.94	
CHL					0.66°					
TOC										-0.68
TSS					-0.78		0.74			0.73

* Blank space indicates not significant at α=0.05.

° Significant at α=0.10.

inversely correlated with one water chemistry parameter, TS. This correlation along with the ratio TS/chlorophyll *a* show that chlorophyll *a* was not a dominant component of the solids in Lake Apopka during the sampling period. In a frequently mixed system such as Lake Apopka, the proportion of total solids which may be attributed to phytoplankton biomass will fluctuate considerably. Other contributors to TS include, bacteria, suspended sediment, zooplankton and inorganic and organic compounds. The inverse relationship between chlorophyll *a* and TS may be interpreted as follows, 1) increased herbivory by high zooplankton populations and 2) light limitation because of high suspended solids.

A peak in TSS was observed in February, which corresponds to the highest wind speed recorded 1 m above the water surface, and thus reflects wind induced sediment resuspension. In a shallow lake, a large proportion of particulate matter within the water column may frequently be attributed to sediment resuspension. The sediments have high P concentrations (Reddy and Graetz 1990) and resuspension will result in increased levels of TP within the water column, TP concentrations were positively correlated with wind speed (5 m above the surface). Conversely, TKN concentrations were inversely related to wind speed (1 m above the surface), and are mainly associated with the algal biomass.

The rate of P exchange between water and sediment increases during suspension of sediment (Pomeroy et al. 1965). Part of this exchange may be biological. Sediment resuspension into the oxygenated water column results in aerobic mineralization of organic P (Lee et al. 1977). Sediments exhibit significant phosphatase activity (Kobori and Taga 1979b, Ayyakannu and Chandramohan 1971) and a positive correlation between SRP released and APA in the water column has been observed during sediment resuspension to the overlying water (Degobbis et al. 1994). Assuming resuspended sediment were contributing significantly to the predominately particulate APA pool in Lake Apopka, the ratio of APA/TSS is a better measure of enzyme activity than APA alone. Comparing monthly means, total APA/TSS was positively correlated with soluble APA (r=0.99) and inversely correlated with TOC (r=-1.00). The correlation between APA and TSS was only observed when data were compared on a site basis. The overall lack of correlation between TSS and APA in this study may be due to 1) insufficient sampling during periods of sediment resuspension, 2) the relationship is hidden by the variability in other parameters incorporated in the TSS pool, and 3) sediment resuspension does not contribute to APA activity.

Alkaline phosphatase activity has been significantly correlated with ATP (Pettersson 1980), particulate organic matter (Gage and Gorham 1985) and chlorophyll *a* (Healey and Hendzel 1979a, Pettersson 1980). In this study, total APA was significantly correlated with chlorophyll *a* in December and inversely correlated with TOC in February. Comparing annual means, there is a strong inverse relationship between total APA and TOC. This may be explained by examining the components of the TOC pool, one contributor is humic material. Alkaline phosphatase activity can be inhibited by high concentrations of humic materials (Francko 1986). The inverse relationship observed between APA and TOC could be a result of binding of APA to organic material. Attachment of alkaline phosphatase enzymes to particulate matter may decrease activity, but may also increase longevity of the enzyme activity (Burns 1986).

Resuspension of sediment high in organic matter could bind enzymes and/or release sediment bound APA to the overlying water column. Organic inputs, living or dead should be considered when measuring APA (Healey and Hendzel 1980)

Soluble APA is positively related to Secchi (r=0.97) suggesting that there is a relationship between APA and water quality. But the low proportion of APA observed in the soluble pool suggest that free dissolved enzymes from cell lysis (Berman 1979) and anzymes excreted by zooplankton (Wynne and Gophen 1981) were not as important as particulate associated APA in the system. Organic phosphorus mineralization is mainly be achieved by APA bound to particulate matter. Examining the data from all sites (excluding site 1), APA was infrequently related to chlorophyll *a*. The overall lack of correlation between APA and chlorophyll *a* may be hidden due to the frequent mixing of the lake water. The particulate nature of APA as determined by the size fractionation scheme corresponds to the chlorophyll *a* distribution. The correlation between APA and chlorophyll *a* leads to the expression of APA as a ratio, i.e., APA/chlorophyll *a* (Pettersson 1980). This ratio tends to increase with P limitation and decrease with trophic state (Pick 1987). Combining data from numerous studies, Pettersson (1999) determined that a ratio between 0.2 to 0.7 nmol APA μg chlorophyll a^{1} min^{1} could be usnd to indicate P limitation. When ambient lake specific APA was consistently < 0.3 nmol APA μg chlorophyll a^{1} min^{1} ratios greater than this were determined to indicate P limitation, i.e., elevated specific APA indicates P limitation (Istvánovics et al 1990) In this study a ratio of < 0.3 nmol APA μg chlorophyll a^{1} min^{1} was consistently observed, suggesting that plankton in Lake Apopka are generally not P limited. This conclusion tends to agree with other research findings from this lake (Aldridge, F. J., personal communication, Department of Fisheries and Aquaculture, University of Florida, Gainesville, FL.; Reddy and Graetz 1990). Specific APA was lowest when chlorophyll a peaked and SRP concentrations of 5 µg L^{-1} were sufficient to support growth.

High particulate APA has been attributed to the location of alkaline phosphatase in the cell wall of phytoplankton (Kuenzler and Perras 1965). Recent research has suggested that viable phytoplankton do not contribute much to the particulate APA pool (Stewart and Wetzel 1982). Lake Apopka is generally dominated by cyanobacteria, with large numbers of *Lyngbya* sp. and *Microcystis* sp. (Shannon and Brezonik 1972; Stites, D. L., personal communication, St. John's River Water Management District, Palatka, FL.) whose mucilaginous layers can support significant bacterial populations. It is likely that particulate APA is attributable to both phytoplankton and the associated bacteria. Use of specific APA (APA/chlorophyll a) to indicate P limitation of phytoplankton should be verified via nutrient enrichment bioassays.

Diel fluctuations in APA were observed, but these do not correspond to any particular water chemistry parameter. This may be attributed to spatial patchiness and water movement (Berman 1970; Wynne 1991). Unlike TKN concentrations, APA did not settle out of the water column following wind subsidence. Diel variability of APA may be dependent upon the species composition of the phytoplankton biomass. In diel studies neither *Thalassiosira pseudonana* Hasle and Heimdal (Perry

1976), nor *Selenastrum capricornutum* Prinz (Klotz 1985b) exhibited diel responses. However, Smith and Kalff (1981) have shown that growth demands for P are more important than the species composition in determining APA.

Preliminary studies (data not shown) and April data demonstrated a strong relationship between APA and TP. Consequently TP, which would include cellular P, would indicate the P forms utilized by APA. However, this relationship was not observed the entire year. Total soluble P, has been suggested as a good indicator of bioavailable P in eutrophic lakes (Bradford and Peters 1987). Correlations between total and soluble APA and TSP were apparent in December, and at certain sites (Table 2-3). Both positive and negative relationships were observed. Total and soluble APA are apparently influenced by different parameters at different sites (Table 2-3, Huber et al. 1985).

In October, the P fractionation experiment demonstrated that APA was inversely related to the acid hydrolyzable P fractions. This suggests that APA may be regulated by acid hydrolyzable compounds, or alternatively they could be used as substrates for APA. Acid hydrolyzable P represents the condensed polyphosphates, a storage form of P in phytoplankton and bacteria. Surplus P concentrations within algal cells have been shown to regulate the production of APA (Fitzgerald and Nelson 1966, Lien and Knutsen 1973, Rhee 1973) and this pool is composed of polyphosphates (Rhee 1972, 1973, Elgavish and Elgavish 1980). Hence, the measurement of surplus P combined with APA could provide a better understanding of the system. Another component which may contribute to the understanding of APA is the concentration of

EHP. No EHP was found in Lake Apopka during the October fractionation experiment. However, this does not reflect the absence of these substrates. In some lakes the release rate of P from EHP satisfies the P uptake rate (Chróst and Overbeck 1987) while in others a large discrepancy exists between these two rates (Heath 1986, Boavida and Heath 1988). Low EHP concentrations have been attributed to the rapid hydrolysis of this fraction (Berman 1970; Taft et al. 1977). Alternatively, this may reflect methodological problems, 1) the method to measure EHP requires the addition of extracted APA from *Escherichia coli* to filtered lake water. This enzyme was not adapted to this system and consequently may not be as efficient or may require a longer incubation time, 2) filtered lake water does not represent the entire P pool available, as particulate organic P, a large portion of TP, may also be susceptible to enzymatic hydrolysis (Jansson 1977), 3) enzymes added from *E. coli* were more inhibited by inorganic P additions than natural enzyme populations (Chróst et al. 1986). The first and third problems were resolved by measuring the increase in SRP in filtered water without the addition of the enzyme (Chróst et al. 1986). However, in a lake which has high particulate APA, this would not be a true representation of the potential APA.

Conclusions

Seasonal and spatial differences in water chemistry were observed. In general, seasonal variability was greater than spatial variability. The system was highly productive as evidenced by chlorophyll a concentrations > 150 µg L^{-1}, and an annual mean of 81 µg L^{-1}. Annual means for TP and TKN were 210 $\mu g\ L^{-1}$ and 4.8 $mg\ L^{-1}$, respectively, confirming the highly eutrophic state of the lake. Conversely, SRP concentrations were consistently < 10 $\mu g\ L^{-1}$.

Alkaline phosphatase activity was mainly associated with particulate matter and was dependent on different water chemistry parameters both seasonally and spatially. In general, APA was not correlated to chlorophyll *a*. The relationship between these parameters may be hidden as a result of the frequent mixing of the water column in Lake Apopka. Both positive and negative correlations between P and APA were observed. An inverse relationship existed between acid hydrolyzable P and APA, indicating polyphosphates may be controlling APA.

The particulate association of APA would suggest that APA should be correlated with TSS, but this was rarely observed. This may be due to the variability in the composition of the TSS pool. The relationship between APA and particulate may be both beneficial and detrimental. Binding to particles results in increased longevity of the enzyme, but it also may inhibit APA by binding to the active site, as indicated by the inverse relationship between APA and TOC.

Future research should examine the components of the TSS pool, plankton and sediment and their effect on organic P mineralization under controlled conditions.

CHAPTER 3
RESPONSE OF NATURAL PLANKTON POPULATIONS TO NUTRIENT ENRICHMENT

Introduction

Soluble inorganic P is the main form of P utilized directly by plankton. Phytoplankton growth rates close to maximal have been determined in the apparent absence of inorganic P (Fuhs et al. 1972, Smith and Kalff 1981). Methods used to measure soluble inorganic P are for the most part limited in sensitivity (Rigler 1956, Tarapchak et al. 1982). This has resulted in the use of physiological indicators to determine the nutritional status of plankton. Information is obtained through a variety of methods including the determination of P uptake rates (Lean and White 1983, Rigler 1956), the measurement of surplus P concentrations (Fitzgerald and Nelson 1966, Rhee 1973) and the determination of alkaline phosphatase activity (APA) (Berman 1970, Kuenzler and Perras 1965). As external inorganic P concentrations decline, plankton are able to utilize internal pools of surplus P to maintain growth (Fitzgerald and Nelson 1966, Rhee 1972, 1973, 1974, Wynne and Berman 1980). Once this internal source of P has been reduced to a critical level some phytoplankton produce phosphatase enzymes, which hydrolyze organic P compounds to inorganic P, to satisfy nutritional demands (Chróst and Overbeck 1987, Kuenzler and Perras 1965, Reichardt 1971). An inverse relationship between APA and surplus P has been reported (Fitzgerald and Nelson 1966, Chróst and Overbeck 1987). Inorganic P is a competitive inhibitor of APA (Coleman and Gettins 1983, Moore 1969, Reid and Wilson 1971). Upon replenishment of external inorganic P concentrations enzyme activity is inhibited (Lien and Knutsen 1973; Torriani 1960, Perry 1972) and surplus P accumulates (Rhee 1973). Surplus P is measured as hot water extractable P (HEP), and in conjunction with APA has been shown to accurately assess P demand in some lakes (Sproule and Kalff 1978, Pettersson 1980) but not in others (Wynne and Berman 1980). Wynne and Berman (1980) observed that the HEP concentration in Lake Kinneret, Israel, remained stable throughout the year even under conditions of P stress and concluded that HEP was a metabolic intermediate rather than a form of P storage.

Lake Apopka is a hypereutrophic lake with soluble reactive P (SRP) concentrations frequently < 1 $\mu g\ L^{-1}$. In contrast, concentrations of total soluble P (TSP) and APA are high (chapter 2). The objectives of this study were to determine whether high APA in Lake Apopka was due to high demand for P or P limitation, and to evaluate the N and P requirements of native plankton.

Materials and Methods

Site Description

Lake Apopka is a 12,500 ha lake located in central Florida (28° 37′ N. latitude, 81° 37′ W. longitude). It has a mean depth of 2 m. It has been proposed that the nutrient loading from the surrounding agricultural and urban areas has precipitated the current hypereutrophic conditions in the lake (USEPA 1979).

Sampling Procedures

Water samples were collected 30 cm below the water surface from the west side of the lake on November 16 1989, and from the east side on April 18 and August 21 1990 (Fig. 3-1). Water was stored in polycarbonate and polyethylene carbays in the dark and at ambient laboratory temperature, for no more than 24 h prior to the start of the experiments.

Experimental Design

The effect of inorganic phosphorus concentrations upon alkaline phosphatase activity

Lake water collected in November 1989 was diluted 2:1 (260 mL unfiltered:140 mL filtered) with filtered lake water (0.45 μm), to reduce the chlorophyll *a* concentration. Four hundred mL were placed in each of 15 wide mouth 500 mL erlenmeyer flasks. The experimental design was completely randomized with 5 treatments and 3 replicates. The water was spiked with nutrient additions (Table 3-1). Nitrogen was added at an N:P ratio of 10:1 to avoid N limitation. The flasks were capped with cotton wool and placed on magnetic stir plates, under a black plastic enclosure in the greenhouse. Temperature within the enclosure was maintained using window air conditioning units and fans (mean ± 1SE,25°C ± 0.21). Light was supplied at 200 μmol photons m^{-2} s^{-1} using cool-white fluorescent lamps. The light:dark schedule was 16:8. The flasks were shaken and aliquots were withdrawn by syringe from treatments 1 and 2 at 0, 24, 72 and 96 h. Aliquots were removed from remaining treatments at 24, 72, and 168 h. Additional sampling times of 260 and 312 h were included for cultures which received 1000 μg L^{-1}. Samples requiring

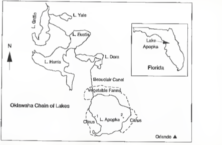

Fig 3-1 Map showing the location of Lake Apopka and sampling sites Water was collected from site 1 in November 1989, and from site 2 in April and August 1990

Table 3-1. Nutrient additions made to diluted lake water collected in November 1989.

Treatment number	Nutrient addition	
	N*	P
	----------$\mu g\ L^{-1}$------------	
1	0	0
2	500	0
3	0	10
4	1000	100
5	2500	1000

[*] N and P were added as potassium nitrate and potassium dihydrogen phosphate, respectively

filtration were immediately filtered through 25 mm membrane filters (0.45 µm) in polypropylene holders which attached to the syringes (Gelman). The samples were analyzed for chlorophyll *a*, total and soluble APA, total (TP), total Kjeldahl N (TKN), SRP, NH_4-N, and $[NO_3 + NO_2]$-N

Nutrient enrichment of natural plankton populations

Experiment 1. Whole lake water was diluted 1:1 with filtered lake water (0.45 µm) and 400 mL were placed in each of 15 wide mouth 500 mL erlenmeyer flasks. The basic experimental design was a 2^2 factorial with an additional 2 fold addition of both N and P included (Table 3-2). The flasks were stoppered with sponge plugs and placed in a clear glass circulating water bath maintained at ambient lake temperature (27°C). The flasks were illuminated from below at an irradiance of 145 µmol photons m^{-2} s^{-1}. The contents of the flasks were mixed daily and immediately prior to sampling. Two h after the nutrient addition, aliquots were withdrawn by syringe at predetermined intervals and analyzed for total APA, SRP, NH_4-N, $[NO_3 + NO_2]$-N, TSP and chlorophyll *a*. Samples requiring filtration were filtered immediately as described above. Hot water extractable P (HEP-SRP) was determined at the beginning and conclusion of the experiment. To obtain a sufficient sample size, HEP-SRP was determined on composite samples containing all treatment replicates.

Experiment 2. To both confirm and compare the results with a different plankton population, a second experiment similar to that described above was conducted in August. Water samples were collected

Table 3-2. Nutrient additions made to diluted lake water collected in April and August 1990.

Treatment	Nutrient addition N*	 P	Incubation temperature
	------------ $\mu g\ L^{-1}$ ------------		°C
Experiment 1-April			
1	0	0	27
2	400	0	27
3	0	40	27
4	400	40	27
5	800	80	27
Experiment 2-August			
1	0	0	29
2	400	0	29
3	0	40	29
4	400	40	29
5	800	80	29
6	0	0	19
7	0	40	19

[*] N and P were added as potassium nitrate and potassium dihydrogen phosphate, respectively.

from the same site, diluted and placed in water baths as described above. The temperature of the bath was set at 25°C to emulate ambient lake water temperature. Another water bath with cultures receiving no nutrient addition and P only was maintained at 19°C, to determine the effect of temperature on the measured parameters. Nutrient uptake rates were determined by measuring the disappearance of the nutrient from solution. During the first 2 h following nutrient addition, the cultures were sampled every 1/2 h and analyzed for SRP, NH_4-N, and $[NO_3 + NO_2]$-N (actual sampling times were 0, 30, 67, 98 and 140 min). Immediately following filtration aliquots were analyzed for SRP. Samples to be analyzed for NH_4-N and $[NO_3 + NO_2]$-N samples were acidified with concentrated H_2SO_4 and stored at 4°C prior to analysis. Samples taken at 0 and 2 h were also analyzed for HEP-SRP, and hot water extractable TSP (HEP-TSP). Aliquots were subsequently removed at 24, 48 and 96 h and analyzed for SRP, NH_4-N, $[NO_3 + NO_2]$-N, total APA, TSP, HEP-SRP, HEP-TSP and chlorophyll *a*.

Analytical Methods

Alkaline phosphatase activity was determined fluorometrically (Healey and Hendzel 1979a). One half mL of substrate, 3-o-methylfluorescein phosphate (Sigma Chemicals), at a concentration determined to be non-limiting, in 0.1 N Tris buffer (Tham, Fisher Scientific) pH 9.6, was added to 4.0 mL of lake water in a cuvette. Both total (whole lake water) and soluble (filtered through 0.45 µm Gelman membrane filter) APA were determined. The cuvettes were placed in a water bath (25°C). At timed intervals during a 20 min period the cuvettes were placed in the fluorometer and fluorescence was measured. The enzyme activity was measured as an increase in fluorescence as the substrate was enzymatically hydrolyzed to the fluorescent product. Fluorescence units were converted to enzyme activity using a standard calibration curve of 3 o-methylfluorescein (Sigma Chemicals). The fluorescence was measured using a Sequoia Turner fluorometer Model 110, equipped with Turner lamp no. 110-953, in combination with 47 B excitation and 2A-12 emission filters. Autoclaved lake water with substrate added was used as a control.

Chlorophyll *a* was determined by measuring *in vivo* fluorescence, using a Turner Design Model 10 fluorometer equipped with a Turner lamp no. 110-953, in combination with 5-60 excitation and 2-64 emission filters. A Sequoia Turner fluorometer Model 110 equipped with the same light source and filters was used to measure chlorophyll *a* fluorescence in the first experiment. Pheophytin *a* fluoresces at the same wavelength as chlorophyll *a*, so chlorophyll *a* concentrations are uncorrected for pheophytin. The calculation of chlorophyll *a* was based on equations by Lorenzen (1967),

$$\frac{\text{ABS}}{89} \times \frac{(\text{vol extracted (mL)})}{(\text{vol filtered (mL)})} \times (\text{unit of measure factor}) \times \frac{1}{\text{pathlength (cm)}}$$

ABS = absorbance at 664 nm

89 = absorption coefficient for chlorophyll *a* in 90% acetone

Unit of measure factor = 10^3 for $\mu g\ L^{-1}$

Initial and final chlorophyll *a* concentrations were calibrated against chlorophyll *a* concentrations determined spectrophotometrically following extraction with 90% acetone (APHA 1985).

Total P, TSP, SRP, NH_4-N, and (NO_3 + NO_2)-N were determined by standard methods (APHA 1985).

Hot water extractable P was determined in experiment 1 using a modification of the method by Fitzgerald and Nelson (1966). Water samples (50 to 100 mL) were filtered through 47 mm 0.45 µm membrane filters (Millipore). The filters were placed in 60 mL borosilicate boiling tubes, and 20 mL deionized water were added. The tubes were capped and autoclaved at 120°C for 1 h. Upon cooling samples were analyzed for SRP. Blank filters were autoclaved to determine any filter contribution to P analysis.

Hot water extractable P includes both a molybdate reactive form of P and a non-reactive form of P. Both hot water extractable forms were determined in experiment 2, using the method of Krausse and Sheets (1980). Nine mL of sample were filtered though 25 mm 0.45 µm membrane filters (Gelman). The filters were placed in 15 mL polypropylene tubes, and 13 mL of deionized water was added. The tubes were capped and autoclaved at 120°C for 1 h. Filter blanks were also autoclaved. Gelman filters were used instead of Millipore filters because they had a lower background P concentrations. The filtrate was analyzed for SRP, and also digested via persulfate oxidation and analyzed for TSP.

Statistical Methods

Data were analyzed using SAS (Statistical analysis systems) version 6. Balanced data (equal number of observations for each treatment) were analyzed using the repeated measures procedure which accounts for the within replicate correlation over time, due to repeated sampling from the same flasks. Unbalanced data (unequal number of observations per treatment) were analyzed using a split plot design with time as the subplat.

Results

The Effect of Inorganic Phosphorus Concentrations upon Alkaline Phosphatase Activity

The majority of N, P and APA were associated with particulate matter (Table 3-3). Due to chlorophyll *a* analysis problems arising from fluorometer calibration, only chlorophyll *a* data from 0 and 72 h are presented and used for statistical analysis (Table 3-4). Increased chlorophyll *a* concentrations were observed at 72 h in all cultures except those which received no nutrient addition. Increased growth was associated with higher nutrient additions, a 69% increase in chlorophyll *a* was observed in treatment 5 (N=2500 P=1000) cultures. Chlorophyll *a* increases of 33% for treatment 4 (N=1000 P=100) and 11 % for treatments 2 (N=500 P=0) and 3 (N=0 P=10) cultures were observed. The opposite was observed for both total and soluble APA (Fig. 3-2a and 3-2b). Total and soluble APA decreased significantly over time in cultures receiving the highest P additions (100 and 1000 µg L^{-1}). No decrease in total APA was observed for treatments 1 (N=0 P=0) and 3 (N=0 P=10), but soluble APA

Table 3-3. Initial concentrations of selected parameters measured in diluted lake water prior to nutrient addition (triplicate samples) in November 1989 (mean ± 1 SE)

Parameter	Concentration
Chlorophyll ($\mu g\ L^{-1}$)	31 ± 6
TP ($\mu g\ L^{-1}$)	66 ± 4
SRP ($\mu g\ L^{-1}$)	3 ± 0.3
TKN ($mg\ L^{-1}$)	3.30 ± 0.1
NH_4-N ($mg\ L^{-1}$)	0.36 ± 0.08
[NO_3 + NO_2]-N ($mg\ L^{-1}$)	0.14 ± 0.00
Total APA ($nM\ min^{-1}$)	13.5 ± 0.3
Soluble APA ($nM\ min^{-1}$)	2.2 ± 0.13

Table 3.4. Chlorophyll *a* and specific alkaline phosphatase activity measured in natural plankton populations collected in November 1989, 72 h after receiving nitrogen and phosphorus additions. 1=no nutrient addition, 2=500 µg N L^{-1}, 3=10 µg P L^{-1}, 4=1000 µg N L^{-1} and 100 µg P L^{-1}, 5=2500 µg N L^{-1} and 1000 µg P L^{-1}.

Treatment	Chlorophyll	Specific APA
	µg L^{-1}	nmol APA µg chlorophyll *a*$^{-1}$ min^{-1}
1	36 a*	0.41
2	40 b	0.46
3	40 b	0.35
4	48 c	0.15
5	61 d	0.03

* Numbers in a column followed by the same letter are not significantly different at $\alpha = 0.05$.

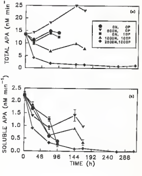

Fig. 3-2. Time courses of alkaline phosphatase activity following nutrient enrichment of natural plankton populations collected in November 1989. 0N,0P=no nutrient addition; 500N,0P=500 μg N L^{-1}, 0N,10P=10 μg P L^{-1}, 1000N 100P=1000 μg N L^{-1} and 100 μg P L^{-1}, and 2500N,1000P=2500 μg N L^{-1} and 1000 μg P L^{-1}. a) total alkaline phosphatase activity, b) soluble alkaline phosphatase activity. Vertical bars represent 1 SE. No vertical bar indicates SE is smaller than symbol size.

did decrease. A significant increase in total APA was observed in cultures which received only N additions suggesting the onset of P limitation. This is more apparent when total APA data are presented as specific activity (i.e. total APA/chlorophyll a) (Table 3-4). A 15 fold difference between specific activity of those cultures which received treatment 5 (N=2500 P=1000) and treatment 2 (N=500 P=0) was observed. Initial specific APA was 0.44 nmol APA μg chlorophyll a^{-1} min^{-1}, hence P addition resulted in a decrease in specific APA. Significant decreases in SRP concentrations were observed within 24 h (Fig. 3-3a). Apart from treatment 5 (N=2500 P=1000), SRP concentrations were the same in all cultures after 2 h. After an initial rapid uptake from 1000 to 673 μg P L^{-1} within the first 2 h, SRP concentrations in cultures treated with 1000 μg P L^{-1} remained constant until 168 h, and then began to decrease. At 312 h, 377 μg P L^{-1}, 37.6% of the original concentration remained in treatment 5 (N=2500 P=1000) cultures.

Nitrate concentrations also exhibited significant treatment differences within 24 h (Fig. 3-3b). The concentrations at 24 h were ranked in descending order of original addition, with 1 and 3 treatments having equivalent (NO$_3$ + NO$_2$)-N concentrations. In all cultures there was a distinct decrease over time. A similar trend was observed for NH$_4$-N, concentrations appeared to decrease within 24 h (Fig. 3-3c), however, no significant differences were determined.

Nutrient Enrichment of Natural Plankton Populations

Growth. Chlorophyll a concentrations and APA were lower in April than in August (Table 3-5). Conversely, TP and TSP were

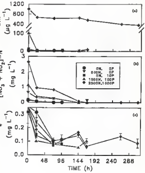

Fig. 3-3. Time courses of nutrient concentrations following nutrient enrichment of natural plankton populations collected in November 1989. 0N,0P=no nutrient addition, 500N,0P=500 μg N L^{-1}, 0N,10P=10 μg P L^{-1}, 1000N,100P=1000 μg N L^{-1} and 100 μg P L^{-1}, and 2500N,1000P=2500 μg N L^{-1} and 1000 μg P L^{-1}. a) soluble reactive phosphorus, b) $[NO_3 + NO_2]$-N, c) NH_4-N. Vertical bars represent 1 SE. No vertical bar indicates SE is smaller than symbol size.

Table 3-5. Initial concentrations of selected parameters measured in diluted lake water prior to nutrient addition (triplicate samples) (mean ± 1 SE).

Parameter	Sampling period	
	April 1990	August 1990
Chlorophyll $\underline{a}$ ($\mu g\ L^{-1}$)	22.5 ± 0	37.4 ± 0
TP ($\mu g\ L^{-1}$)	113 ± 12	56 ± 9
TSP ($\mu g\ L^{-1}$)	70 ± 10	9 ± 0
SRP ($\mu g\ L^{-1}$)	1 ± 0	2 ± 0
HEP-TSP ($\mu g\ L^{-1}$)	ND*	33 ± 3◆
HEP-SRP ($\mu g\ L^{-1}$)	7 ± 0♠	15 ± 2◆
NH_4-N ($mg\ L^{-1}$)	0.04 ± 0	0.05 ± 0
[NO_3 + NO_2]-N ($mg\ L^{-1}$)	0 ± 0	0.61 ± 0
Total APA ($nM\ min^{-1}$)	9.1 ± 0.2	12.7 ± 0.1

* ND indicates data not determined

◆ Determined by the method of Krausse and Sheets (1980)

♠ Determined by the method of Fitzgerald and Nelson (1966) significantly higher in April. Chlorophyll *a* was used as a means to indicate phytoplankton growth in the cultures. Chlorophyll *a* concentrations increased in response to nutrient additions to the water samples collected in both April and August. Although small and hidden due to axis scale, significant treatment differences in chlorophyll *a* occurred within the first 24 h (Fig. 3-4 and 3-5a). After 96 h, maximum chlorophyll *a* concentrations in April were 99 µg L^{-1} and exceeded 180 µg L^{-1} in August. In April, chlorophyll *a* concentrations were significantly affected by the interaction between concentrations of N and P added. Chlorophyll *a* concentrations obtained in the presence of both nutrients exceeded those obtained by single nutrient additions (Fig. 3-4). The greatest initial increase in chlorophyll *a* occurred in cultures which received treatment 2 (N=400 P=0). Subsequently, growth rates in treatments 4 (N=400 P=40) and 5 (N=800 P=80) exceeded those of treatment 2. This information, combined with the lag in growth observed in cultures receiving only P additions, suggest that phytoplankton were initially N limited and became co-limited by P as they grew. This is confirmed by the significant interaction of added N and P levels upon chlorophyll *a* concentrations. In contrast, no significant interaction between the levels of N and P was observed in August. In August, those cultures which received only P additions had the same chlorophyll *a* as those which received both N and P, while cultures receiving only N had the same chlorophyll *a* as those with no nutrient addition. Increases in chlorophyll *a* were also recorded in cultures which received no P addition. These observations suggest that some P was still available for plankton growth, but the growth rates were P limited, and thus the

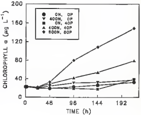

Fig 3-4 Time courses of chlorophyll *a* concentrations following nutrient enrichment of natural plankton populations collected in April 1990. 0N,0P=no nutrient addition; 400N,0P=400 µg N L^{-1}, 0N,40P=40 µg P L^{-1}; 400N,40P=400 µg N L^{-1} and 40 µg P L^{-1}, and 800N,80P=800 µg N L^{-1} and 80 µg P L^{-1}. Vertical bars indicate ± SE. No vertical bar indicates SE is smaller than symbol size.

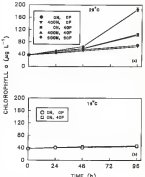

Fig. 3-5. Time courses of chlorophyll *a* concentrations following nutrient enrichment of natural plankton populations collected in August 1990. 0N,0P=no nutrient addition; 400N,0P=400 µg N L^{-1}, 0N,40P=40 µg P L^{-1}; 400N,40P=400 µg N L^{-1} and 40 µg P L^{-1}, and 800N,80P=800 µg N L^{-1} and 80 µg P L^{-1}. a) plankton grown at 29°C, b) plankton grown at 19°C. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size.

growth rate increased with increasing P concentration in the growth media. It is suggested that cultures were only slightly limited by P, because cultures with no P enrichment still increased in biomass. During both experiments, the highest nutrient addition (treatment 5, N=800 P=80) resulted in significantly greater chlorophyll a than any other treatment.

Growth of plankton collected in August was inhibited at cooler temperatures (Fig. 3-5b). Over the entire 96 h period chlorophyll a only increased by 9 μg L^{-1}. The phytoplankton grown at 19°C with either a P addition or no nutrient addition only achieved 65% of the chlorophyll a of phytoplankton grown at 29°C with no nutrient addition.

Nutrient uptake. In April, the initial sampling for nutrient analyses occurred after 2 h; however, at this time SRP concentrations had been significantly reduced, consequently to give a true representation of the data a time 0 was included along with 2 h to the data set. Time 0 represents the means of initial concentrations measured in cultures with no nutrient addition, plus the respective additions, hence approximate uptake rates can be envisioned (Fig. 3-6). Only the cultures which received treatment 5 (N=800 P=80) had significant SRP concentrations remaining after 2 h (17 μg L^{-1}). SRP concentrations in the other cultures were below 3 μg L^{-1}. These concentrations remained close to baseline for the remainder of the experiment. The determination of P uptake thus required a more intensive sampling immediately following nutrient addition. This was achieved in experiment 2, with water samples collected in August. Within 30 min SRP had decreased from 91.6 to 37.4 μg L^{-1} in treatment 5

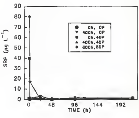

Fig 3-6. Time courses of soluble reactive phosphorus concentrations following nutrient enrichment of natural plankton populations collected in April 1990. 0N,0P=no nutrient addition; 400N,0P=400 μg N L^{-1}; 0N,40P=40 μg P L^{-1}; 400N,40P=400 μg N L^{-1} and 40 μg P L^{-1}, and 800N,80P=800 μg N L^{-1} and 80 μg P L^{-1}. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size

(N=800 P=80), from 41.6 to 22 $\mu g\ L^{-1}$ in treatment 4 (N=400 P=40) and from 41.6 to 13.1 $\mu g\ L^{-1}$ in treatment 3 (N=0 P=40) cultures (Fig. 3-7a). Uptake rates were calculated as the disappearance of SRP within the first 30 min (Table 3-6). This was selected to indicate maximal uptake because the slope changed over time as the P demand decreased (Fig. 3-7a). Cultures which received 80 $\mu g\ L^{-1}$ had a significantly greater uptake rate than those which received 40 $\mu g\ L^{-1}$. As expected, temperature had a significant effect upon SRP uptake (Fig. 3-7b). The uptake was not as rapid as that for the same treatment at 29°C ($p = 0.12$), but even with the 10°C difference in temperature all SRP had been depleted to below detection within 2 h. After 1 h, SRP levels in treatments 3 (N=0 P=40) and 4 (N=400 P=40) were no longer significantly different. The SRP concentrations continued to decrease in all the cultures and were all close to baseline in 2 h and were undetectable (<1 $\mu g\ L^{-1}$) after 24 h. In both experiments TSP decreased within the first 2 h and then remained constant (Fig. 3-8a and 3-9a).

The uptake of N differed between the two sampling periods. In April, [NO_3 + NO_2]-N concentrations in the cultures decreased. The concentration decreased by 25% in all treatments with N additions, within 2 h and continued to decrease over time (Fig. 3-8b). In contrast, NH_4-N concentrations did not change in any of the cultures until 216 h, when an increase was observed in control and N cultures (Fig. 3-8c). In August, no significant treatment by time interaction was recorded for [NO_3 + NO_2]-N, and no apparent uptake of [NO_3 + NO_2]-N occurred (Fig. 3-9b). A significant treatment by time interaction was

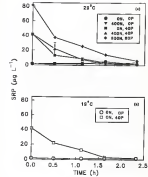

Fig. 3-7. Time courses of soluble reactive phosphorus concentrations following nutrient enrichment of natural plankton populations collected in August 1990. 0N,0P=no nutrient addition, 400N,0P=400 µg N L^{-1}, 0N,40P=40 µg P L^{-1}, 400N,40P=400 µg N L^{-1} and 40 µg P L^{-1}, and 800N,80P=800 µg N L^{-1} and 80 µg P L^{-1}. a) plankton grown at 29°C, b) plankton grown at 19°C. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size.

Table 3-6 Phosphorus uptake rates for natural plankton populations collected in August 1990, 30 min after receiving nitrogen and phosphorus additions (mean ± SE). 3=40 $\mu g\ P\ L^{-1}$, 4=400 $\mu g\ N\ L^{-1}$ and 40 $\mu g\ P\ L^{-1}$, 5=800 $\mu g\ N\ L^{-1}$ and 80 $\mu g\ P\ L^{-1}$ 7=40 $\mu g\ P\ L^{-1}$ and plankton grown at 19°C.

Treatment	Uptake Rate
	$\mu g\ P\ L^{-1}\ min^{-1}$
3	0.95 ± 0.05 a[*]
4	0.43 ± 0.27 b
5	1.47 ± 0.06 c
7	0.65 ± 0.01 ab[•]

[*] Numbers in a column followed by the same letter are not significantly different at $\alpha = 0.05$

[•] Uptake rates for treatments 3 and 7 are significantly different at $\alpha = 0.12$

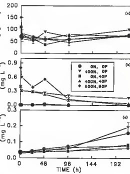

Fig 3.8. Time courses of nutrient concentrations following nutrient enrichment of natural plankton populations collected in April 1990. 0N,0P=no nutrient addition; 400N,0P=400 µg N L^{-1}; 0N,40P=40 µg P L^{-1}; 400N,40P=400 µg N L^{-1} and 40 µg P L^{-1}, and 800N,80P=800 µg N L^{-1} and 80 µg P L^{-1}: a) total soluble phosphorus; b) [NO_3 + NO_2]-N; c) NH_4-N. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size.

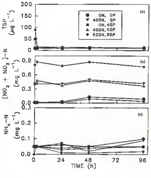

Fig. 3.9. Time courses of nutrient concentrations following nutrient enrichment of natural plankton populations collected in August 1990 grown at 29°C. 0N,0P=no nutrient addition, 400N,0P=400 μg N L^{-1}, 0N,40P=40 μg P L^{-1}, 400N,40P=400 μg N L^{-1} and 40 μg P L^{-1}, and 800N,80P=800 μg N L^{-1} and 80 μg P L^{-1}. a) total soluble phosphorus, b) $[NO_3 + NO_2]$-N, c) NH_4-N. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size.

observed for NH_4-N, however, the data were highly variable, making meaningful interpretation difficult (Fig. 3-9c). Plankton grown at 19°C exhibited the same responses for TSP, [NO_3 + NO_2]-N, and NH_4-N (data not shown).

Surplus phosphorus. Soluble P extracted from plankton following boiling with deionized water (HEP-SRP) was used as an indicator of surplus P. In April HEP-SRP was determined at both the start and conclusion of the experiment. Hot water extractable P decreased in cultures which did not receive P additions but remained the same in cultures which received 40 µg L^{-1} (Table 3-7). Those cultures which received 80 µg P L^{-1} had almost a 3-fold increase in HEP-SRP after 216 h. The role of HEP was examined in more detail in water samples collected in August. Seventy percent of initial TP was accounted for by HEP-TSP and TSP (Table 3-5), with HEP-TSP representing 59% of TP. Within 2 h of P addition, substantial increases in HEP-TSP were observed (Fig. 3-10a). Hot water extractable-TSP tripled from 33.3 to 99.3 µg P L^{-1} upon addition of 80 µg P L^{-1}. Addition of 40 µg P L^{-1} resulted in a doubling of HEP-TSP to 65 and 69 µg P L^{-1} in cultures which received treatments 3 (N=0 P=40) and 4 (N=400 P=40), respectively. Even with a 10°C temperature difference HEP-TSP accumulated to 63.9 µg P L^{-1} within 2 h (Fig. 3-10b). Cultures grown at 19°C which did not receive nutrients had greater HEP-TSP concentrations after 2 h than those with no nutrient addition grown at 29°C. At 29°C the HEP-TSP remained constant within 24 h and then decreased by 15 µg P L^{-1} for P added treatments. The downward trend continued to 96 h, HEP-TSP

Table 3-7. Hot water extractable phosphorus concentrations of composite lake water samples collected in April 1990, 216 h after nutrient additions. 1=no nutrient addition, 2-400 µg N L^{-1}, 3-40 µg P L^{-1}, 4-400 µg N L^{-1} and 40 µg P L^{-1}, 5=800 µg N L^{-1} and 80 µg P L^{-1}.

Treatment	Hot water extractable SRP
	µg L^{-1}
1	3.0
2	2.8
3	7.2
4	6.8
5	18.4

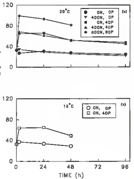

Fig. 3-10. Time courses of hot water extractable total soluble phosphorus following nutrient enrichment of natural plankton populations collected in August 1990. 0N,0P=no nutrient addition;400N,0P=400 µg N L^{-1}; 0N,40P=40 µg P L^{-1}; 400N,40P=400 µg N L^{-1} and 40 µg P L^{-1}, and 800N,80P=800 µg N L^{-1} and 80 µg P L^{-1}. a) plankton grown at 29°C. b) plankton grown at 19°C. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size

concentrations in treatment 1 (N=0 P=0) cultures were lower than initial concentrations while HEP-TSP concentrations in treatments 3 (N=0 P=40) and 4 (N=400 P=40) were higher than initial concentrations Normalizing the data to chlorophyll *a*, a decrease in HEP-TSP was observed for all treatments following the initial increase at 2 h (Table 3-8). The final ratios were lower than the initial ratios.

The treatment by time response was different for HEP-SNP (Fig 3-11a) The relative increases were greater An increase in HEP-SNP concentrations was observed after 48 h in cultures which received P, except for treatment 4 (N=400 P=40) Hot water extractable P in treatments 1 (N=0 P=0) and 2 (N=400 P=0) cultures remained constant. A significantly lower increase was observed in treatment 7 (N=0 P=40, temperature=19°C) (Fig 3-11b α=0 06). Normalizing the data to chlorophyll *a* resulted in a continuous decline in HEP-SNP No increase after 48 h was observed (Table 3-9). A significant correlation between HEP-SNP and chlorophyll *a* was observed (r=0 56).

Alkaline phosphatase activity. Considerable differences in the production of APA were observed between April and August (Fig 3-12a and 13a) In both experiments, increases in APA were observed, however in August this increase only lasted 48 h for all cultures grown at 28°C In April, APA in cultures receiving both N and P additions was inhibited within 2 h, 28% and 11% inhibition for treatments 5 (N=800 P=80) and 4 (N=400 P=40), respectively No inhibition was observed in treatments receiving only a P addition After initial inhibition, which lasted 24 h, APA increased (Fig 3-12a). A significant interaction between N and P was apparent at 48 h. Cultures receiving only P had a delayed

Table 3-8. Specific hot water extractable phosphorus measured over time in natural plankton populations collected in August 1990 after receiving nitrogen and phosphorus additions (mean ± 1 SE). 1=no nutrient addition, 2=400 μg N L^{-1}, 3=40 μg P L^{-1}, 4=400 μg N L^{-1} and 40 μg P L^{-1}, 5=800 μg N L^{-1} and 80 μg P L^{-1}, and 6=no nutrient addition and 7=40 μg P L^{-1} and plankton grown at 19°C.

	Time h				
Treatment	0	2	24	48	96
	μg P μg chlorophyll $\underline{a}^{-1}$				
1	0.89 ± 0.07	0.73 ± 0.04	0.68 ± 0.02	0.51 ± 0.02	0.36 ± 0.02
2		0.92 ± 0.03	0.67 ± 0.04	0.51 ± 0.02	0.33 ± 0.02
3		1.75 ± 0.03	1.34 ± 0.03	0.89 ± 0.04	0.45 ± 0.01
4		1.85 ± 0.06	1.25 ± 0.03	0.90 ± 0.04	0.44 ± 0.02
5		2.66 ± 0.05	1.82 ± 0.01	1.25 ± 0.04	NA*
6		1.00 ± 0.07	0.82 ± 0.02	0.72 ± 0.07	NA
7		1.71 ± 0.01	1.59 ± 0.03	1.10 ± 0.03	NA

* NA indicates data is not available.

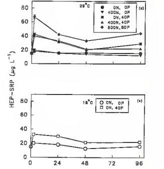

Fig 3-11 Time courses of hot water extractable soluble reactive phosphorus following nutrient enrichment of natural plankton populations collected in August 1990. ON,OP=no nutrient addition;400N,OP=400 µg N L^{-1}, ON,40P=40 µg P L^{-1}, 400N,40P=400 µg N L^{-1} and 40 µg P L^{-1}, and 800N,80P=800 µg N L^{-1} and 80 µg P L^{-1}. a) plankton grown at 28°C. b) plankton grown at 18°C. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size.

Table 3-0 Specific hot water extractable phosphorus measured over time in natural plankton populations collected in August 1990 after receiving nitrogen and phosphorus additions (mean ± 1 SE): 1=no nutrient addition, 2=400 µg N L^{-1}, 3=40 µg P L^{-1}, 4=400 µg N L^{-1} and 40 µg P L^{-1}, 5=800 µg N L^{-1} and 80 µg P L^{-1}, and 6=no nutrient addition and 7=40 µg P L^{-1} and plankton grown at 19°C.

Treatment	Time h				
	0	2	24	48	96
	µg P µg chlorophyll $\underline{a}^{-1}$				
1	0.41 ± 0.05	0.40 ± 0.07	0.34 ± 0.01	0.20 ± 0.01	0.22 ± 0.00
2		0.53 ± 0.01	0.35 ± 0.02	0.27 ± 0.00	0.10 ± 0.01
3		1.14 ± 0.00	0.04 ± 0.04	0.35 ± 0.01	0.20 ± 0.02
4		1.07 ± 0.06	0.67 ± 0.06	0.37 ± 0.01	0.21 ± 0.02
5		1.79 ± 0.07	0.02 ± 0.03	0.51 ± 0.02	0.24 ± 0.00
6		0.54 ± 0.02	0.44 ± 0.01	0.31 ± 0.04	0.35 ± 0.05
7		0.47 ± 0.05	0.73 ± 0.01	0.40 ± 0.01	0.47 ± 0.03

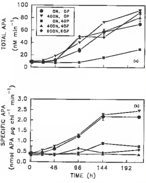

Fig. 3-12 Time courses of alkaline phosphatase activity following nutrient enrichment of natural plankton populations collected in April 1990. ON,OP=no nutrient addition, 400N,OP=400 µg N L^{-1}, ON,40P=40 µg P L^{-1}, 400N,40P=400 µg N L^{-1} and 40 µg P L^{-1}, and 800N,80P=800 µg N L^{-1} and 80 µg P L^{-1}. a) total alkaline phosphatase activity, b) specific alkaline phosphatase activity. Vertical bars indicate ±1 SE. No vertical bar indicates SE is smaller than symbol size.

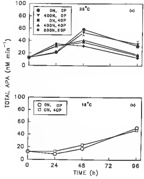

Fig 3-13. Time courses of alkaline phosphatase activity following nutrient enrichment of natural plankton populations collected in August 1990. 0N,0P=no nutrient addition, 400N,0P=400 μg N L^{-1}; 0N,40P=40 μg P L^{-1}, 400N,40P=400 μg N L^{-1} and 40 μg P L^{-1}, and 800N,80P=800 μg N L^{-1} and 80 μg P L^{-1}. a) plankton grown at 29°C; b) plankton grown at 19°C. Vertical bars indicate 1 SE. No vertical bar indicates SE is smaller than symbol size.

response in APA increase and produced the least APA. A similar trend was observed by phytoplankton cultured at 19°C in August (Fig. 3-13b). In August the response of cultures grown at 29°C with only P added mimicked those which received both N and P (Fig. 3-13a). Disregarding temperature effects, APA in cultures with and without P additions exhibited similar trends; increasing up to 48 h and then decreasing (Fig. 3-13a). The initial increase in APA over the first 24 h was on average 64%, greater in those cultures receiving P. The reverse was true for the next 24 h period. Alkaline phosphatase activity measured in treatments 1 (N=0 P=0) and 2 (N=400 P=0) doubled while only a 22% increase was observed in treatments 3 (N=0 P=40) and 4 (N=400 P=40). Cultures which received treatment 5 (N=900 P=80) did not exhibit a change. Both groups subsequently declined by approximately 20 nM min^{-1} to 96 h. Transforming the APA data to specific activity results in a different shape curve for April data (Fig. 3-13b) but no change in curve shape in August (Table 3-10). The interpretation from both experiments is the same, i.e., higher specific APA was apparent in all cultures which did not receive any P addition. While those cultures which received P had significantly lower specific APA. The highest specific APA was recorded in April in cultures which received treatments 1 (N=0 P=0) and 2 (N=400 P=0).

Discussion

Nutrient loading from external and internal sources can influence the productivity of phytoplankton and other aquatic biota. The response of phytoplankton growth to nutrient enrichment has been used as an

Table 3-10 Specific alkaline phosphatase activity measured over time in natural plankton populations collected in August 1990 after receiving nitrogen and phosphorus additions (mean ± 1 SE) 1=no nutrient addition, 2=400 μg N L^{-1}, 3=40 μg P L^{-1}, 4=400 μg N L^{-1} and 40 μg P L^{-1}, 5=800 μg N L^{-1} and 80 μg P L^{-1}, and 6=no nutrient addition and 7=40 μg P L^{-1} and plankton grown at 19°C

	Time h			
Treatment	0	24	48	56
	---------- nmol APA μg chlorophyll a^{-1} min^{-1} ----------			
1	0.34 ± 0.00	0.47 ± 0.01	1.07 ± 0.03	0.45 ± 0.03
2		0.47 ± 0.02	1.01 ± 0.07	0.93 ± 0.09
3		0.63 ± 0.01	0.94 ± 0.02	0.19 ± 0.02
4		0.63 ± 0.02	0.70 ± 0.03	0.17 ± 0.00
5		0.57 ± 0.02	0.48 ± 0.01	0.07 ± 0.00
6		0.22 ± 0.01	0.43 ± 0.01	1.12 ± 0.03
7		0.32 ± 0.02	0.52 ± 0.01	1.00 ± 0.04

indicator of nutrient status. This study examined growth, uptake rate, surplus P and APA to explain P requirements of phytoplankton and associated microorganisms in Lake Apopka. Natural plankton populations were used to include the contributions of bacteria and zooplankton to the overall nutrient status of the lake. The objective was to determine the response of phytoplankton biomass, hence chlorophyll a measurements were used to indicate biomass.

This study demonstrated that APA is immediately and rapidly inhibited by high inorganic P concentrations (Garen and Levinthal 1960, Moore 1969; Torriani 1960). The extent of inhibition was dependant upon the concentration of inorganic P added, internal P concentrations and the growth of the plankton (Fitzgerald 1969). The most severe inhibition was caused by 1000 µg P L^{-1}. It has been suggested that 1000 µg P L^{-1} is the minimum requirement for APA inhibition (Jones 1979b).

In combination with external P concentrations, the extent of APA inhibition may also be dependent upon the initial internal nutrient content. In April, APA was inhibited by 40 and 80 µg P L^{-1} within the first 2 h but these concentrations did not result in such rapid inhibition in August. Phytoplankton growth as determined by increases in chlorophyll a concentrations, were initially limited by N and had sufficient P so additions of P resulted in inhibition of APA. As the phytoplankton grew and exhausted internal supplies, growth became limited by both N and P, and APA increased. In August, phytoplankton were P limited, thus the demand was sufficient that even the addition of 80 µg P L^{-1} did not inhibit APA. Initial APA was also higher in August than April, and may reflect the difference between slight P limitation and initial N limitation. Alternatively, the difference in response may be due to different algal populations which have phosphatases with different affinities (Pettersson 1980). In April, the algal population (on a biovolume basis) was dominated by *Microcystis* sp. and pennate diatoms. In August, the population was dominated by *Microcystis* sp. and *Lyngbya contorta* (Aldridge, F. J., personal communication, Department of Fisheries and Aquaculture, University of Florida, Gainesville, FL.). Smith and Kalff (1981) showed that APA is influenced more by the equilibration of plankton growth with nutrient supply, rather than the species composition of the plankton population.

The P demand by plankton populations in Lake Apopka was very high as shown by the rapid uptake of SRP in August. These rates are higher than those obtained by similar methods from other lakes, i.e., 0.02 to 0.03 μg P L^{-1} min^{-1} (Rigler 1956) and 0.017 to 0.43 μg P L^{-1} min^{-1} uptake rates (Lean and White 1983). Within the first 2 h of P enrichment, an increase in HEP-SRP equivalent to 66% of inorganic P added was observed. The HEP-TSP increase accounted for 98% of added inorganic P. A 10°C reduction in temperature decreased percentage uptake to 43 and 78% as HEP-SRP and HEP-TSP, respectively. The concentrations of HEP-TSP decreased as algal biomass increased. At 96 h an increase in HEP-SRP was observed for cultures which received treatments 3 (N=0 P=40) and 5 (N=900 P=90). Internal P concentrations have been shown to regulate APA (Chróst and Overbeck 1997; Fitzgerald and Nelson 1966; Moore 1969). The increase at 96 h thus could account for the decreased APA. In April, 1990 HEP-SRP concentrations were shown to be inversely related to APA, while in August a strong inverse relationship was not recorded in response to treatment, suggesting that internal P levels were not sufficiently low to control APA. Comparing specific HEP-SRP (HEP-SRP/chlorophyll *a*) values in April, P limited cultures had ratios < 0.09 µg P^{-1} µg chlorophyll *a*$^{-1}$ while treatments 3 (H-0 P-40) and 5 (H-800 P-80) had ratios > 0.12 µg P L^{-1} µg chlorophyll *a*$^{-1}$. Specific HEP-SRP ratios obtained after 96 h in August were generally > 0.2 µg P L^{-1} µg chlorophyll *a*$^{-1}$. In August, after 96 h, an increase in HEP-SRP occurred at the same time as an increase in growth rate for treatments 3 and 5. High growth rates in P limited *Scenedesmus* sp. were associated with increased surplus P (Rhee 1974). No increase in HEP-SRP at 96 h was observed in the cultures which received treatment 4. This may be anomalous because measurements of both HEP-TSP and APA obtained from treatment 4 cultures agree with those determined in treatment 3 cultures. Increased HEP-SRP concentrations may not be the only factor regulating growth and P limitation, because decreased APA was also observed in the other cultures. Stable low concentrations of HEP-SRP, with rapidly declining specific APA and uptake rates, combined with increasing chlorophyll *a*, have been suggested to indicate that P is immediately utilized for growth as opposed to P storage (Sproule and Kalff 1978). An inverse relationship between specific APA and growth rate has been recorded (Smith and Kalff 1981). Phosphorus required for growth is probably provided from the hydrolysis of small chain polyphosphates from the continually decreasing HEP-TSP concentrations. While HEP-TSP concentrations declined, no increase in any other P parameter measured was sufficient to account for the disappearance of HEP-TSP. Hence it appears that HEP-TSP was utilized metabolically, and supports the suggestion of Wynne and Berman (1980), that HEP-TSP can be utilized for nutrition.

It was interesting to note that HEP-TSP concentrations increased within 2 h in cultures which were not enriched with P. Phytoplankton have been shown to grow even at SRP concentrations of $< 3\ \mu g\ L^{-1}$ provided this concentration is maintained at the cell surface (Fuhs et al. 1972). Hence, although SRP concentrations were low and did not change, phytoplankton may have utilized P at the cell surface. In conjunction with this, SRP concentrations were at the limit of detection and not sensitive enough to record further decreases. Thus, it is apparent that the monitoring of P limitation responses would provide a more accurate assessment of the nutritional status of the plankton.

Alkaline phosphatase activity has been shown to increase with eutrophication, in combination with increased plankton biomass (Gorham and Gage 1985, Jones 1972b). Attempts to associate APA with specific parameters has resulted in the normalization of APA to protein (Stevens and Parr 1977), particulate organic matter (Gage and Gorham 1985) and ATP (Healey and Hendzel 1980; Pettersson 1980). Specific APA (i.e. normalized to chlorophyll *a*) has frequently been used as a measure of P limitation and tends to decrease with trophic status (Pick 1987). Specific APA values of 0.2-0.7 nmol APA μg chlorophyll *a*$^{-1}$ min^{-1} under field conditions and values > 0.08 nmol APA μg chlorophyll *a*$^{-1}$ min^{-1} obtained in laboratory cultures have been suggested to indicate P limitation (Pettersson 1980, Healey and Hendzel 1979a). In this study, initial specific APA (0.3-0.4 nmol APA μg chlorophyll *a*$^{-1}$ min^{-1}) would thus indicate that this system is P limited. In April, specific APA of plankton which received P additions remained between 0.3-0.4 nmol APA µg chlorophyll $\underline{a}^{-1}$ min^{-1} for the first 96 h. Hence this ratio may represent constitutive APA (Gage and Gorham 1985). Specific APA determined in a eutrophic Swedish lake remained at < 0.3 nmol APA µg chlorophyll $\underline{a}^{-1}$ min^{-1} for most of the year but increased to 0.8 nmol APA µg chlorophyll $\underline{a}^{-1}$ min^{-1} during periods of P limitation (Pettersson et al. 1980). Specific APA thus increases with the severity of P limitation (Perry 1976). In this study plankton grown in the absence of P produced specific APA > 1 nmol APA µg chlorophyll $\underline{a}^{-1}$ min^{-1}. Greater fluctuation in specific APA was apparent in August. However, comparing specific APA with increases in chlorophyll $\underline{a}$, in general, specific APA measured in cultures which received P was inversely related to growth rate (Rhee 1973, Smith and Kalff 1981). Specific APA determined bimonthly at 7 sites in Lake Apopka from April 1989 to February 1990, was < 0.3 nmol APA µg chlorophyll $\underline{a}^{-1}$ min^{-1}, this suggests that growth of Lake Apopka plankton was not severely P limited. This agrees with results from other nutrient limitation studies conducted on Lake Apopka (Aldridge, F. J., unpublished data, Department of Florida, University of Florida, Gainesville, FL.). Hence, it would appear that the expression of APA and HEP relative to chlorophyll $\underline{a}$ is an accurate indicator of the nutrient status of plankton in Lake Apopka. However, it should be re-emphasized that APA and HEP are found in numerous organisms, phytoplankton, bacteria and zooplankton which vary both spatially and temporally, absolute values may vary. Some uncertainty could be removed by normalizing the data to ATP, and thus express the indicator relative to living biomass (Healey and Hendzel 1980).

Although the focus of this research has been on P limitation, Lake Apopka has been found to often be N limited or co-limited by both N and P (Aldridge, F. J., unpublished data, Department of Fisheries and Aquaculture, University of Florida, Gainesville, FL.). The uptake of NO_3-N, shown to be the preferred form of N for Lake Apopka phytoplankton (Aldridge, F. J., unpublished data), gives an insight into the N requirements of the system. In April, a situation of co-limitation, $[NO_3 + NO_2]$-N concentrations were shown to decrease over time, and NH_4-N concentrations tended to increase over time, suggesting that mineralization of organic N occurred. In August, when plankton were established as being slightly P limited, no decrease in $[NO_3 + NO_2]$-N concentrations were observed.

The limitation of the analyses used in this study is that APA and HEP concentrations do not differentiate between P limitation and co-limitation. Nitrogen limitation parameters, such as ammonium enhancement, should also be determined to assess the N requirements of the plankton and thus enable the assessment of N limitation in conjunction with P limitation (Vincent et al. 1984).

The continued measurement of HEP-SRP as a P limitation indicator has been questioned based on its highly dynamic nature (Wynne and Berman 1980; Cembella 1984b). Under ambient conditions such rapid accumulation of HEP-SRP may not occur (Wynne and Berman 1980). Others have shown that HEP-SRP is a good indicator of the nutritional status of plankton (Pettersson 1980, Sproule and Kalff 1978). In this study it appeared to be a reliable indicator of the nutritional status of the plankton

Conclusions

Results from this study show that APA of Lake Apopka plankton is inhibited by high concentrations of inorganic P. Hence, ambient lake water SRP concentrations (<10 μg L^{-1}) will not be sufficient to inhibit APA. Both P limitation and co-limitation of N and P were observed.

During conditions of inorganic P limitation, plankton P uptake demand was very high as shown by the rapid uptake of SRP. Uptake rates as high as 1.5 μg L^{-1} min^{-1} were reported. The first step in the metabolism of the added P was the accumulation of internal P as identified by hot water extraction. After the apparent removal of all SRP from the growth medium, plankton utilized P from the HEP-SRP and HEP-TSP pools for growth. Studies assessing the importance of surplus P frequently only determine HEP-SRP, however, this study highlighted the need to measure both HEP-TSP and HEP-SRP.

Alkaline phosphatase activity tended to increase with growth of the plankton, however, the intensity of APA was dependent upon the conditions of P limitation. Severe P limitation was indicated by specific APA values > 1 nmol APA μg chlorophyll $\underline{a}^{-1}$ min^{-1}. Specific APA values < 1 nmol APA μg chlorophyll $\underline{a}^{-1}$ min^{-1} may indicate slight P limitation, but APA is associated with numerous organisms in this eutrophic system, which change both spatially and temporally, hence P limitation should be confirmed by nutrient enrichment bioassays.

CHAPTER 4
THE EFFECT OF SEDIMENT RESUSPENSION ON ALKALINE PHOSPHATASE ACTIVITY

Introduction

In many lakes, exchange of P between the sediment and the water column is dependent upon diffusion related processes (Stumm and Leckie 1971, Tessenow 1972). However, in shallow lakes, P exchange also occurs due to sediment resuspension during wind events which increase the interaction between the sediment and the overlying water column. It has been estimated that the upper 10 cm of sediment is actively involved in exchange reactions as a result of resuspension with the overlying water column (Tessenow 1972, Schindler et al. 1977). However, the amount of sediment that will mix with the water column is a function of the shear stress and sediment type (Lee, 1970).

The immediate result of sediment resuspension is the increase in suspended solids concentration in the water column. The suspended sediment has been shown to provide 28-41% algal available P (Dorich et al. 1985), as well as physically transporting soluble P to the water (Ryding and Forsberg 1977). Sediment resuspension also results in increased exchange of soluble reactive P (SRP) from the sediment to the water column (Holdren and Armstrong 1980, Pollman 1983). The increase in exchange has been associated with biological activity (Pomeroy et al. 1965). Conversely, SRP may be removed from the water column as a result of sorption to the particulate material (Gächter and Mares 1985, Reddy and Fisher 1990).

Resuspension results in increased aeration of the sediments. Under aerobic conditions P release from sediments has been associated with the decomposition of organic matter (Lee et al. 1977). If the sediments are dominated by Fe, aeration results in increased sedimentation of P (McQueen et al. 1986), however, in highly organic sediments, biological processes catalyzed by numerous phosphatase enzymes are likely to dominate (Ayyakkannu and Chandramohan 1971). Organic P mineralization in sediments is regulated by the activity of enzymes such as phosphatases, particularly alkaline phosphatase activity (APA) in sediments at neutral and alkaline pH (Ayyakannu and Chandramohan 1971, Kobori and Taga 1979b). A significant positive correlation between SRP released and phosphatase activity in the water column was observed during resuspension of marine sediments (Degobbis et al. 1984). Thus the level of APA within the sediment will have an effect on the APA subsequently resuspended in the overlying water column.

Phosphatase activity decreases with depth of soils (Juma and Tabatabai 1978, Speir and Ross 1978) and sediments (Degobbis et al. 1984, Kobori and Taga 1979b). This corresponds to a decrease in microbial biomass, C, N and organic P with depth (Juma and Tabatabai 1978, Speir and Ross 1978, Baligar et al. 1988). Consequently the depth of material resuspended is significant in influencing APA in the water column.

Following subsidence of the wind event, suspended solids settle thus transporting any associated material from the water column to the sediment, i.e. APA, and results in a decrease in organic P mineralization within the water column. Settling seston is also a sink for SRP (Gächter and Mares 1985).

The objectives of this study were to determine, 1) the depth distribution of APA in the sediment and the overlying water column and, 2) the effect of sediment resuspension upon SRP and APA release. It was hypothesized that sediment resuspension may affect APA by 1) increasing SRP levels in the water column and competitively inhibiting activity, 2) releasing alkaline phosphatase from the sediment to the water column, and 3) a combination of 1 and 2.

Materials and Methods

Site Description

Lake Apopka is a 12,500 ha hypereutrophic lake, located in central Florida, 28°37′ N latitude, 81°37′ W longitude (Fig. 4-1). It has a mean depth of 2 m. Chlorophyll *a* values exceeding 100 $\mu g\ L^{-1}$ are frequently recorded (chapter 2). Nutrient loading from the surrounding agricultural and urban areas has resulted in the current hypereutrophic conditions in the lake (USEPA 1979). The bottom sediments in the lake have a 30 cm unconsolidated flocculent layer at the surface, underlain by consolidated flocculent material (Reddy and Graetz 1990). The sediments have an alkaline pH, hence the phosphatases of interest are those with maximum activity in the alkaline region, i.e. alkaline phosphatase.

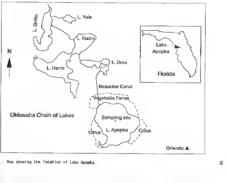

Fig. 4-1. Map showing the location of Lake Apopka.

Field sampling procedures

Water. Water samples were collected on May 23 1989, from the center of the lake (Fig. 4-1.) using an alpha sampler (Wildco), at depths 0, 0.5, 1 and 1.5 m below the surface. Water was stored in 1 L polyethylene bottles kept on ice until return to the laboratory. Dissolved oxygen (DO) and temperature (YSI, Model 58) and pH (Orion, Model SA 230) were recorded with depth. Light penetration was estimated by measuring the Secchi disk transparency. Within 24 h of return to the laboratory, samples were analyzed for total and soluble APA. Other parameters measured were total Kjeldahl N (TKN), total P (TP), SRP, and chlorophyll a.

Sediment. Three intact sediment cores were collected from the deck of a boat in May 1989 from the center of the lake using a 9 cm (I.D.) x 1 m (length) Plexiglass-PVC sediment core sampler (Reddy and Graetz, 1990). The cores were taken to a depth of 40 cm, capped and brought to the laboratory for sectioning. The cores were sectioned at 0-2, 2-5, 5-10, 10-20 and 20-40 cm intervals, placed in 125 mL centrifuge tubes, immediately purged with N_2 and stored at 4°C until analysis. Preliminary studies demonstrated that sediments could be held for at least 3 weeks at 4°C with no change in APA. Alkaline phosphatase activity, porewater SRP, and water content were determined on wet sediment. NaOH-extractable P, an indication of bioavailable inorganic P and labile organic P (Darich et al. 1985; Young et al. 1985) was also measured. Total P, organic P and volatile solids content of dried sediment were determined.

Experimental Design

Experiment 1. Six intact sediment cores were collected by boat from the center of the lake on September 25 1999. The cores were taken to a depth of 30 cm, capped and brought to the laboratory and maintained under ambient light and temperature conditions. Overlying water was siphoned off to leave equal volumes of water in all cores. Previous studies have shown that the concentration of SRP in the porewater of the upper 10 cm sediments remain close to ambient lake water concentrations (Reddy and Graetz 1990). The high water content (98%) associated with the low SRP concentrations of the surface sediments suggest that these sediments are frequently resuspended. Consequently, the surface 10 cm of sediment was resuspended into the overlying water column for 1 h using a sediment resuspension device similar to that described by Wolanski et al. (1989), (Fig. 4-2). This involved the use of a 52 cm long Plexiglas rod to which 15 Plexiglas rings (approximately 1 cm thick) were attached at 2 cm intervals. The rod was oscillated within the core above the sediment surface. The depth of placement of the rod was adjusted so that only the surface 10 cm of sediment was resuspended. The surface sediments in three cores were resuspended and the remaining three cores were left undisturbed as controls. During resuspension a composite 70 mL sample was withdrawn by syringe from the resuspended material (time =1 h). At predetermined intervals, 70 mL composite samples were withdrawn from the water column of all cores. Water samples were analyzed for total and soluble APA, SRP, total soluble P (TSP), TP, TKN and total suspended solids (TSS). At the end of the 24 h sampling period, the surface 10 cm of sediments of the cores

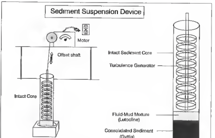

Fig. 4-2 Diagram of the sediment resuspension device (Source: Reddy and Fisher 1990)

were sectioned into 2.5 cm intervals, placed in 125 mL centrifuge tubes, immediately purged with N_2 and stored at 4°C until analysis. The sediments were analyzed for total APA, TP, organic P and porewater SRP.

Experiment 2. A second experiment was conducted in January 1990 to further evaluate the effect of varying depths of bottom sediment resuspension upon APA and associated parameters within the water column. Twelve sediment cores were collected on January 23 1990, to a depth of 20 cm. The overlying water was siphoned off to leave a 45 cm water column. There were three treatments, the top 2, 5 or 10 cm of triplicate cores were resuspended for 15 min using the procedures described in experiment 1. There were 3 replicates for each treatment and 1 undisturbed core to serve as a control. At predetermined intervals, 70 mL water samples were withdrawn. The amount of resettling was measured and the water withdrawn from the midpoint of the exposed water. Alkaline phosphatase activity, TP, TKN, SRP, TSS and total organic carbon (TOC) of the water column were determined. At the end of the sampling period, the surface 2, 5 and 10 cm sediment fractions representing the respective treatments were sectioned in resuspended and control cores, and analyzed as described above. In addition, APA was determined in the porewater using the method described for total sediment APA, to determine whether the activity was predominantly particulate or soluble.

Analytical Methods

Water. Alkaline phosphatase activity was determined fluorometrically (Healey and Hendzel 1979a). One half mL of substrate, 3-o-methylfluorescein phosphate (Sigma Chemicals), at a concentration determined to be non-limiting, in 9.1 M Tris buffer (Thme, Fisher Scientific) pH 9.6, was added to 4.0 mL of lake water in a cuvette. Both total (whole lake water) and soluble (filtered through 0.45 µm Gelman membrane filter) APA were determined. The cuvettes were placed in a water bath (25°C). At timed intervals during a 20 min period the cuvettes were placed in the fluorometer and the fluorescence measured. The enzyme activity was measured as an increase in fluorescence as the substrate was enzymatically hydrolyzed to the fluorescent product. Fluorescence units were converted to enzyme activity using a standard calibration curve of 3-o-methylfluorescein (Sigma Chemicals). The fluorescence was measured using a Turner fluorometer No. 110, equipped with Turner lamp no. 110-853, in combination with 47 B primary and 2a-12 secondary filters. Autoclaved lake water with substrate added was used as a control.

Chlorophyll a was determined spectrophotometrically following extraction with acetone and correction for pheophytin (APHA (1002 G), 1995). Total P, TSP, TKN, TOC, SRP and TSS were determined by standard methods (APHA 1995).

Sediments. Alkaline phosphatase activity was determined by a method adapted from Sayler et al. (1979). Sediment samples (1 g wet sediment) or 1 mL of porewater, were placed in centrifuge tubes. Three mL of 1 M Tris-Tris HCl buffer (pH 7.6) were added to each tube. The average pH found in Lake Apopka sediments was approximately 7.6. The samples were sonicated (Heatsystems Ultrasonics Model

W-220F) for 45 s at 30% relative output, to release cell bound phosphatase. The samples were then incubated with 1 mL of 50 mg mL^{-1} of *p*-nitrophenyl phosphate, (Sigma Chemicals) at 25° C for 1 h. After 1 h, 3 mL of 1 M NaOH were added to the tubes to stop the reaction and enhance *p*-nitrophenol color. Controls to account for substrate color and color release during solubilization of organic matter by the NaOH, were obtained by incubating sediment without the substrate and subsequently adding the substrate along with NaOH at the end of the incubation. All samples were then centrifuged at 7000 rpm (7096 g) for 15 min. The liquid was removed and absorbance at 410 nm (Shimadzu Model UV-160) measured. Concentrations were determined by calibration with a standard curve of *p*-nitrophenol (Sigma Chemicals).

Porewater was extracted by centrifuging the sediment subsample under anaerobic conditions at 5000 rpm (3620 g) for 15 min. The porewater was immediately filtered through 0.45 µm Gelman membrane filters. Soluble reactive P was measured using standard methods (APHA 1985). Sodium hydroxide extractable P was obtained by shaking 5 g of wet sediment with 20 mL of 0.1 M NaOH for 16 h. Total P and SRP of filtered extracts were then measured using standard methods described for water.

Water content was determined by drying a known weight of wet sediment at 70°C to a constant weight. The dried sediment was ground to pass through a 20 mesh screen, using a Spex 8000 grinding mill. Volatile solids were reported as the loss in weight due to ignition of dried sediment at 500°C for 2 h. Total and organic P content of the dried sediment was determined via ignition (Walker and Adams 1958).

Results

Physico-chemical Properties

Water. Nutrient concentrations were evenly distributed throughout the water column (Table 4-1). Chlorophyll *a*, temperature, DO, and pH decreased with depth. Total APA in the water column increased with depth from a surface concentration of 37 to 44 nM min^{-1} at 1.5 m. Soluble APA was <2% of total APA. A high concentration of soluble APA was measured at a depth of 1 m.

Sediment. Total APA in the sediments decreased with depth, with the greatest change occurring within the 20-40 cm depth (Fig. 4-3a). The water content of the sediments decreased from 98% at the surface to 95% in the 20-40 cm depth (data not shown). The porewater SRP remained constant within the first 0-20 cm but increased significantly within the 20-40 cm depth from 0.01 mg L^{-1} to 1.4 mg L^{-1} (Fig. 4-3b). Volatile solids also increased at this depth (Fig. 4-3c). The opposite effect was observed for NaOH-extractable P, organic P and TP, which decreased in the 20-40 cm sediment depth (Fig. 4-4a, b, and c).

Sediment Resuspension Effects on Alkaline Phosphatase Activity

Experiment I

Water column. Resuspension of the top 10 cm of sediment resulted in increased TSS from 60 to 3000 mg L^{-1}. Within 30 min after resuspension, most of the sediment particles settled. Concentrations of all parameters (excluding TSP) increased in the water column during resuspension (time 1 h) and decreased to initial concentrations within 24 h (Table 4-2). Soluble parameters, e.g. SRP, exhibited considerable

Table 4-1 Distribution of selected parameters measured in May 1989, within the water column at the center of Lake Apopka (n=3)

Depth	APA Total	APA Soluble	TKN	TP	SRP	DO	pH	Chl a	Temp	Secchi
m	--nM min^{-1}--		mg L^{-1}	--µg L^{-1}--		mg L^{-1}		µg L^{-1}	°C	m
0	37.2	0.6	6.26	210	3	6.5	9.13	96	27.5	0.25
0.5	40.5	0.6	5.56	270	4	6.4	6.14	66	27.0	
1.0	43.6	1.5	6.66	270	4	6.7	6.08	66	26.3	
1.5	43.6	6.3	7.43	260	4	5.7	6.66	83	26.3	

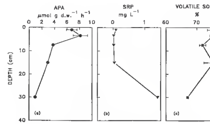

Fig 4-3 The depth distribution of selected parameters measured in triplicate sediment cores collected in May 1989 from the center of Lake Apopka: a) alkaline phosphatase activity, b) porewater soluble reactive phosphorus, c) volatile solids. Bars indicate 1 SE. No bar indicates SE is smaller than symbol size.

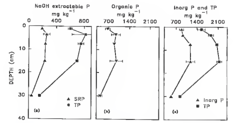

Fig. 4-4 The depth distribution of selected parameters measured in triplicate sediment cores collected in May 1989 from the center of Lake Apopka: a) NaOH extractable phosphorus; b) organic phosphorus; c) total and inorganic phosphorus. Horizontal bars indicate 1 SE. No bar indicates SE is smaller than symbol size.

Table 4-2. Concentrations of parameters measured within the water column of cores collected in September 1988, from the center of Lake Apopka (mean ± 1 SE)

Treatment	Time*	TKN	TP	TSP	SRP	APA Total	APA Soluble	TSS
	h	mg L^{-1}	-------- µg L^{-1}		-------	-------- nM min^{-1}	------	mg L^{-1}
Control	0	3.55 ± 0.06	100 ± 13	100 ± 28	11 ± 3	8.8 ± 0.3	0.04 ± 0.00	72 ± 2
	1	3.65 ± 0.12	80 ± 6	80 ± 3	5 ± 0	8.0 ± 3.6	0.08 ± 0.03	76 ± 4
	24	3.89 ± 0.12	150 ± 30	80 ± 10	4 ± 1	6.8 ± 0.3	1.20 ± 0.17	54 ± 3
	48	4.31 ± 0.12	180 ± 50	60 ± 3	5 ± 0	12.5 ± 0.7	0.41 ± 0.06	47 ± 3
Resuspended	0	3.78 ± 0.33	80 ± 3	100'	8 ± 5	5.6'	0.28 ± 0.20	59 ± 1
	1	86.00 ± 5.65	5733 ± 245	70 ± 10	5 ± 1	108.1 ± 3.0	0.07 ± 0.06	3236 ± 38
	24	5.44 ± 0.12	160 ± 23	80 ± 3	4 ± 0	6.1 ± 0.2	0.55 ± 0.08	64 ± 1
	48	4.33 ± 0.55	130 ± 3	70 ± 3	5 ± 1	14.5 ± 0.8	0.15 ± 0.04	70 ± 8

* Time 0 represents samples taken prior to resuspension, 1 h represents end of period of suspension

' Only 1 replicate per measurement

variation with no apparent change in concentration. The undisturbed control sediment cores maintained constant nutrient concentrations throughout the duration of the experiment.

Sediment. The APA in the control sediment cores decreased with sediment depth. No distinct depth profile of APA was observed in the cores where the top 10 cm of sediment was resuspended (Fig. 4-5), although there was a tendency for the APA to be lower in the surface sediment and increase with depth. There was no net change in porewater SRP, TP, organic P and inorganic P concentrations in any of the cores.

Experiment 2

Water. The resuspension of different depths of surface sediment into the overlying water column resulted in distinct differences among measured parameters. Resuspension of the surface 10 cm resulted in TSS values of 3000 mg L^{-1} which decreased to 100 mg L^{-1} within 1 h (Fig. 4-6a). The resuspension of 5 cm of sediment produced TSS values around 1700 mg L^{-1} which decreased to 174 mg L^{-1} within 1 h. This compares to the settling of the 2 cm depth increment which produced a resuspended TSS of 400 mg L^{-1} and decreased more slowly to 140 mg L^{-1} within 1 h. As observed in experiment 1, increases in TP, TKN and TOC corresponded to TSS increases (Fig. 4-6). The total APA also increased with TSS, and declined as the suspended matter settled (Fig. 4-7).

Combining the data from both resuspension experiments, the relationship between total APA and TSS measured within the water column (Fig. 4-8) was best described by the following power equation

Total APA = 0.031 x $(TSS)^{0.865}$, $r^2 = 0.91$ n=32

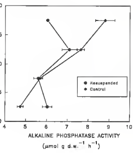

Fig 4-5 The depth distribution of alkaline phosphatase activity in triplicate resuspended and undisturbed (control) sediment cores collected in September 1989 from the center of Lake Apopka. Horizontal bars indicate ± SE. No bar indicates SE is smaller than symbol size.

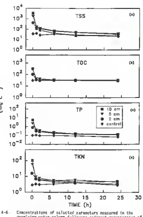

Fig. 4-6. Concentrations of selected parameters measured in the overlying water column following sediment resuspension of 0, 2, 5, and 10 cm surficial sediments: a) total suspended solids, b) total organic carbon; c) total phosphorus; d) total Kjeldahl nitrogen. Initial data point indicates the conclusion of resuspension. Vertical bars indicate ± SE. No bar indicates SE is smaller than symbol size

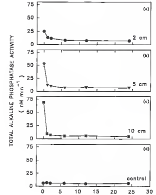

Fig. 4-7. The total alkaline phosphatase activity measured in the overlying water column of triplicate sediment cores after resuspension of surficial sediments: a) 2 cm resuspended, b) 5 cm resuspended, c) 10 cm resuspended, d) control, no resuspension. Initial data point indicates the conclusion of resuspension. Vertical bars indicate 1 SE. No bar indicates SE is smaller than symbol size

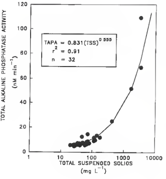

Fig 4-8 The relationship between alkaline phosphatase activity and total suspended solids in the overlying water column of sediment cores after resuspension of surficial sediments.

The soluble APA measured in the water column after resuspension of 2 cm of sediment exhibited the same trend as observed for total APA, decreasing as the TSS decreased (Fig. 4-9). Soluble APA did not show significant differences over time in the cores in which the surface 5 and 10 cm were resuspended.

Soluble reactive P data exhibit such variability that no statistically significant response was observed. However, all cores including undisturbed cores, exhibited a tendency to increase at time 12 h (Fig. 4-10). This would suggest that SRP was slowly desorbed from the underlying sediment.

Sediment. Changes in total and porewater APA within the sediment of control and disturbed cores were not significant (Table 4-3). The highly variable porewater APA accounted for less than 1% of the total activity.

Discussion

The physico-chemical parameters measured within the water column of Lake Apopka showed no distinct stratification, however, DO, pH, temperature and chlorophyll *a* tended to decrease with depth. The relatively high concentrations of nutrients and chlorophyll *a* in the water column are characteristic of hypereutrophic systems, although SRP concentrations are very low. The APA is also representative of productive systems (cf. Heath and Cooke 1975). The soluble APA accounted for only 3% of the total APA, therefore, the majority of APA within this system was associated with particulate matter. A possible incomplete settling of particulate matter would explain the increase in

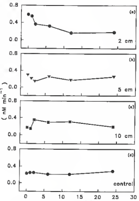

Fig. 4-9 Soluble alkaline phosphatase activity measured in the overlying water column of triplicate sediment cores after resuspension of 0, 2, 5 and 10 cm surficial sediments: a) 2 cm resuspended, b) 5 cm resuspended, c) 10 cm resuspended, d) control, no resuspension. Initial data point indicates the conclusion of resuspension. Absence of vertical bar indicates symbol size is greater than 1 SE.

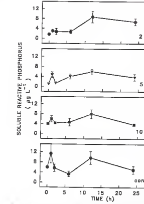

Fig. 4-10. Soluble reactive phosphorus concentrations measured in the overlying water column of triplicate sediment cores after resuspension of 0, 2, 5 and 10 cm surficial sediments: a) 2 cm resuspended, b) 5 cm resuspended, c) 10 cm resuspended, d) control, no resuspension. Initial data point indicates the conclusion of resuspension. Vertical bar indicates 1 SE. Absence of vertical bar indicates symbol size is greater than SE.

Table 4-3. The distribution of alkaline phosphatase activity in disturbed and undisturbed sediment cores collected from the center of Lake Apopka in January 1990 (means ± 1 SE).

	Alkaline phosphatase activity	
	Total	Porewater
	μmol g dry wt^{-1} h^{-1}	μmol L^{-1} h^{-1}
Control		
2 cm	14.0*	0.91
5 cm	15.3	0.22
10 cm	10.5	1.07
Resuspended		
2 cm	17.8 ± 0.2	0.45 ± 0.14
5 cm	14.0 ± 1.5	0.34 ± 0.03
10 cm	9.5 ± 0.5	1.27 ± 0.38

[*] Control sediment cores were not replicated.

APA at the sediment-water interface, while there was no apparent change in soluble APA.

In the sediment, APA was shown to decrease with depth. Soluble reactive P is a competitive inhibitor of APA (Coleman and Gettins 1983), hence the increase of porewater SRP with sediment depth may partially explain the decrease in APA, due to inhibition of the production of the enzyme. Organic P and APA have been shown to be positively correlated (Juma and Tabatabai 1978, Speir and Ross 1978). A high enzyme activity may be maintained in the presence of competitive inhibitors by the presence of increased substrate concentrations; however, the decrease in organic and NaOH extractable P with depth suggests a decrease in substrate concentrations. Alkaline phosphatase activity was also found to have a positive correlation with organic matter (Speir and Ross 1978), therefore the decrease in volatile solids (an indicator of organic matter), may have contributed to the APA decrease. However, the most probable cause for the reduced activity with depth is a decrease in microbial biomass. Decreasing APA with sediment depth has been shown to have a positive correlation with microbial biomass (Sayler et al. 1979, Ayyakkannu and Chandramohan 1971). Microbial biomass was not measured in this study, however, a decrease with depth could be expected in these sediments because of highly reduced (anaerobic) conditions at lower depths in Lake Apopka sediments (Moore et al. 1991). Pulford and Tabatabai (1988) suggested that under anaerobic conditions, the higher solubility of metals such as Fe and Mn results inhibition of APA (Juma and Tabatabai 1978). Lake Apopka sediment is Ca dominated (Moore et al.

1991), thus, inhibition of APA under anaerobic conditions is probably due to reduced microbial numbers and metabolism

The sediment APA observed in this study is in the same range as those reported for other freshwater systems (APA = 6-18 μmol g dry wt.$^{-1}$ h^{-1}) with fine particulate organic matter (Sayler et al. 1979) The APA of marine sediments was much lower, i.e., 0.2-3.3 μmol g dry wt.$^{-1}$ h^{-1} (Ayyakkannu and Chandramohan 1971, Degobbis et al. 1984). However, a comparison of the sediment APA values obtained in other studies is difficult due to the lack of standardization in methodology used. Other complications include the pretreatment of samples, e.g. drying of the soil (Tabatabai and Bremner 1969) which may affect the APA values of some soils (Skujins 1976; Speir and Ross 1978).

Short-term resuspension of surficial sediments increased TSS, TKN, TP and TOC concentrations. This is expected because all these parameters are interrelated The concentrations of these species decreased rapidly following the end of resuspension, as a result of particle settling. However, the soluble fraction, i.e. SRP, did not exhibit significant increases as observed previously (Pollman 1983, Reddy and Graetz 1990) in Lake Apopka cores. The porewater SRP concentrations of the cores used in these resuspension experiments was very low (<5 μg L^{-1}), thus the resuspension of these sediments would not result in an increase in the SRP concentration of the water column. Although the TP concentration in the surficial sediments is high (1200 mg kg^{-1}), a major portion is organically bound and is not readily available. The low porewater SRP concentrations suggest that the surface sediments have a high P sorptive capacity A high C/P ratio of the surface sediments also suggests that microbial immobilization of inorganic P occurs (Reddy and Graetz 1990)

The mechanism of P release from suspended particles will depend on the rate of P desorption from the solid phase to the liquid phase and the physico-chemical properties of the water column. Resuspension has been shown to increase the biological breakdown of organic P (Pomeroy et al. 1965). Resuspension of anaerobic sediments to the overlying oxygenated water column will result in aeration of the sediments. This may make the associated organic matter more susceptible to enzymatic hydrolysis (Pulford and Tabatabai 1988). The pH of the surface sediment was 7, compared to a water column pH of 9-9. Thus any SRP release into the water column upon resuspension could be immediately precipitated as calcium phosphates (Moore et al. 1991).

In shallow lakes sediment resuspension may play an important role in P recycling (Ryding and Forsberg 1977). Due to the association of APA with TSS, prolonged resuspension would result in higher APA levels within the water column. As observed by Burns (1986), the attachment of APA at a non-active site would result in increased longevity of the enzyme within the aquatic system. If phosphomonoesters are present SRP would be released. The insignificant, but apparent gradual increase in SRP in all cores at 12 h, may be due to the enzymatic degradation of the more easily hydrolyzed organic P compounds. Hence, APA may enhance the ability of sediments and particulate matter to recycle P. To test the relationship observed between TSS and total APA, seasonal data collected in the field (chapter 2) was fitted to the equation . A good fit was observed when TSS were high (≈ 100 mg L^{-1}) and chlorophyll *a* concentrations were low. However, with normal TSS ($\approx$ 70 mg L^{-1}) and high chlorophyll *a*, the equation underestimated the measured APA. This suggests that the predictive capability of this equation is only valid during periods of resuspension, when sediment particulate matter is the dominant component of the TSS pool. After 49 h an increase in total APA was observed in the water column of both control and resuspended cores, this may be in response to APA production following P limitation of the plankton population. These increases are apparent as the two points above the regression line in Fig. 4.8. Consequently, during quiescent periods other contributors to the TSS pool, e.g. phytoplankton, should be included to predict APA.

Apart from the cores in which the surface 2 cm was resuspended, soluble APA was not shown to increase due to resuspension. The sediment porewater APA was greater than the water soluble APA, however, the dilution effect due to resuspension brought the porewater concentration to ambient levels. The APA attributed to porewater exhibited high variability. This variability could be partially due to differences between cores, and also the insensitivity of the method at such low APA. The fluorescent technique used for lake water would be more sensitive, however, different substrate specificities have been observed (Pettersson and Jansson 1978). Even considering the variability in the porewater APA, it was clearly demonstrated that >99% of the APA was associated with the solids portion of the sediment. A recent study demonstrated the association of APA with the larger particulate matter (Rojo et al. 1990) and it has been suggested by Burns (1986) that the covalent bonding of enzymes to organic matter would result in increased stabilization of the enzyme.

Conclusions

Most studies investigating APA in aquatic systems have emphasized the contribution of free-living bacteria, phytoplankton and zooplankton. This study demonstrated the contribution of the sediment associated APA to the total APA pool of the water column. Sediment resuspension resulted in increased APA and TP in the overlying water column, and hence there was an increase in potential organic P mineralization. This increased APA remained high only while sediment particles were still suspended in the water column and decreased upon settling of the sediment. This indicates that no APA was released from the sediment into the water column. The APA determined in both sediments and lake water was mainly associated with particulate matter and thus may have increased longevity. The high APA recorded both in the sediment and water columns suggests that organic P mineralization, via APA, may play a significant role in P cycling of Lake Apopka. The importance of this process is dependent upon the concentration of organic substrates. The forms of sediment organic P capable of acting as substrates needs to be evaluated.

CHAPTER 5
THE EFFECT OF SEDIMENT AND WATER COLUMN ANOXIA ON ORGANIC PHOSPHORUS MINERALIZATION

Introduction

Organic P constitutes the major component of total P in the sediment and water column of lakes. When soluble inorganic P concentrations within the water column are low plankton may produce phosphatase enzymes, which hydrolyze organic P compounds with the release of inorganic P (Kuenzler and Perras 1965, Reichardt 1971). As a result the measurement of alkaline phosphatase activity (APA) has been used as a tool to indicate P limitation and potential organic P mineralization through enzymatic hydrolysis (Healey and Hendzel 1979b, Gage and Gorham 1985).

Under well oxygenated conditions (aerobic), mineralization of organic P is rapid. However, depletion of dissolved oxygen (DO) concentrations can occur in the water column as a result of high respiratory activity. Under reduced DO concentrations the rate of enzymatic hydrolysis of organic P compounds could be reduced as the metabolism of aerobic plankton is reduced. This may be of particular importance at the sediment-water interface where DO concentrations are often depleted. Oxygen concentrations in the water column may vary both diurnally (Howeler 1972, Reddy 1981) and seasonally (Mortimer 1941). In rare circumstances, metalimnetic DO depletion may occur with increasing eutrophication (Shapiro 1960) More often, highly productive aquatic systems exhibit clinograde DO distributions, resulting in the depletion of DO with water depth in response to summer stratification (Miyake and Saruhashi 1956, Wetzel 1983) and consumption during high respiratory activity at the sediment-water interface (Charlton 1980, Boström et al. 1982)

Diffusion of DO from the water, bioturbation and sediment resuspension can maintain relatively aerobic conditions at the sediment-water interface Under these conditions P release from sediments to overlying water can be due to mineralization of organic P (Lee et al 1977) Mineralization of organic P may be of particular importance in sediments which possess abundant organic substrates, such as peat sediments (Ayyakannu and Chandramohan 1971)

During stratification of the water column, the hypolimnion may become anoxic, hence the surface sediment becomes anaerobic. A significant consequence of anaerobic conditions at the sediment-water interface is the increase in water soluble P in the overlying water column (Holdren and Armstrong 1980, Ponnamperuma 1972; Mortimer 1941) The release of P under these conditions is the result of the reduction of ferric phosphates to the more soluble ferrous phosphates (Mortimer 1941). This flux of P from the sediment to the overlying water column may have a significant impact upon APA in the water column Inorganic P is a competitive inhibitor of APA (Coleman and Gettins 1983), and sedimentary release of P has been shown to inhibit APA within the overlying water column (Pettersson 1980) Anaerobic conditions may also result in the inhibition of organic P mineralization by APA in the sediment (Pulford and Tabatabai 1988).

Regardless of the oxygen status of the surface sediments, redox potential tends to decrease with sediment depth (Kobori and Taga 1979b, Degobbis et al. 1984). Bacterial populations were also observed to decrease with sediment depth, in response to redox potential changes (Kobori and Taga 1979b). Bacterial numbers have been positively correlated with APA in sediments (Ayyakannu and Chandramohan 1971), hence APA also decreases with depth (Kobori and Taga 1979b). An important effect of reduced Eh conditions may be the accumulation of soluble organic P compounds due to incomplete mineralization. It is apparent that oxygen concentrations both in the sediment and in the water column may play a significant role in determining P mineralization.

The objective of this study was to determine the effects of anoxia upon organic P mineralization in the sediment and water column of a highly productive lake. It is known that APA is a non-specific enzyme capable of hydrolyzing numerous organic P compounds (Coleman and Gettins 1983, Cembella et al. 1984a). However, specific forms of sedimentary organic P are mainly unknown; identification is based upon the susceptibility to different extraction solutions (Bowman and Cole 1978, Sommers et al. 1972). A second objective of this research was to determine the forms of extractable P influenced by different redox conditions, and their effect on the mineralization of organic P by APA

Materials and Methods

Site Description

Lake Apopka is a 12,500 ha hypereutrophic lake, located in central Florida, 28°37′ N latitude, 91°37′ W longitude. It has a mean depth of 2 m. Chlorophyll *a* values exceeding 100 $\mu g\ L^{-1}$ are frequently recorded (chapter 2). Nutrient loading from the surrounding agricultural and urban areas has resulted in the current hypereutrophic conditions in the lake (USEPA 1979) The bottom sediments in the lake have an average of 30 cm unconsolidated flocculent layer at the surface, underlain by consolidated flocculent material (Reddy and Graetz 1990)

Sampling Procedures

Water column Water was collected on July 2 1990, 30 cm below the water surface from the center of the lake in 15 and 30 L polycarbonate containers. The water used for the first experiment was kept for less than 24 h in the dark under ambient laboratory conditions prior to the start of the experiment. The water used to dilute the sediment was first coarse filtered and stored under ambient laboratory conditions in polycarbonate containers until the start of the experiment.

Sediment. Grab samples of the surface 30 cm of sediment were collected on 28 June 1990 from the center of the lake using an Eckman dredge, and placed in polycarbonate containers, which were filled completely to minimize air spaces. The samples were stored under ambient laboratory conditions prior to use in the study.

Experimental Design

The effect of dissolved oxygen on organic phosphorus mineralization in the water column. Dissolved O_2 minima in the water column of lakes occur during the night hours when respiration exceeds photosynthesis. To emulate this condition this experiment was conducted in the dark, under ambient laboratory temperatures. Four hundred mL of lake water were placed in each of six 500 mL erlenmeyer flasks. The flasks were placed on stir plates and continuously stirred. Three flasks were stoppered and maintained under anaerobic conditions (Fig. 5-1a), while three were maintained under aerobic conditions. One replicate of each treatment had pH and DO probes in constant contact with the water column, it was assumed that all three replicates would respond similarly. Anaerobic conditions were obtained by closing the flasks with rubber stoppers and purging with a mix of 330 ppm CO_2 balanced with N_2 for approximately 1 1/2 h until the DO concentration was <0.2 mg L^{-1}. The pH and DO probes were tightly sealed in the stoppers. A rubber septum was also included in the stopper arrangement to allow sampling. At this point 30 mL water samples were removed by syringe from all flasks. A time series of samples was subsequently removed after 2, 4, 8 and 24 h. Samples were analyzed for total and soluble APA, total soluble P (TSP), total soluble N (TSN), NH_4-N, and soluble reactive P (SRP). Initial chlorophyll *a* was also determined.

The effect of redox potential on organic phosphorus mineralization in the sediment. Fresh sediment was diluted with filtered lake water to obtain 10 g dry wt. L^{-1} of slurry, with a total volume of 2.5 L and was placed in each of six 2.8 L flasks, which were

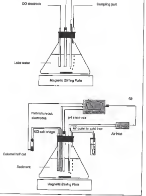

Fig. 5-1 Diagram illustrating the apparatus used to control dissolved oxygen concentration and redox potential: a) anoxic control in lake water, b) redox potential control of sediment suspensions

stoppered, placed on stir plates and attached to redox controllers (Cole Palmer Model 5997-20) (Fig. 5-1b) modified from Patrick et al. (1973). All flasks were purged with a mixture of 330 ppm CO_2 balanced with N_2, for 24 h. The flasks were then allowed to equilibrate for 1 month in the dark under ambient laboratory conditions at the desired redox potentials; -250, -100, 0, +100, +250 and +500 mV (actual potentials measured upon sampling were; -242, -157, -2, +48, +338, +483 mV). After equilibration, triplicate samples were withdrawn from the flasks and analyzed for APA and fractionated for organic P using an adaptation of the method of Sommers et al. (1972) (Fig. 5-2). Bicarbonate extractable P was determined upon sediment samples after the removal of porewater for pH determinations (Fig. 5-2). Total P and volatile solids were measured on dried sediment samples.

Analytical Methods

Water. Alkaline phosphatase activity was determined fluorometrically (Healey and Hendzel 1979a). One half mL of substrate, 3-o-methylfluorescein phosphate (Sigma Chemicals), at a concentration determined to be non limiting, in 0.1 M Tris buffer (Tham, Fisher Scientific) pH 8.6, was added to 4.0 mL of lake water in a cuvette. Anoxic water samples were injected into cuvettes which were sealed by a rubber septum and had been purged with N_2 and then evacuated. Both total (whole lake water) and soluble (filtered through 0.45 µm Gelman membrane filter) APA were determined. The cuvettes were placed in a water bath (25°C). At timed intervals during a 20 min period the

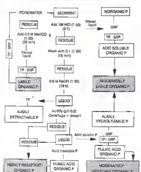

Fig. 5-2. The extraction scheme used to fractionate organic phosphorus in sediment.

cuvettes were placed in the fluorometer and fluorescence was measured. The enzyme activity was measured as an increase in fluorescence as the substrate was enzymatically hydrolyzed to the fluorescent product. Fluorescence units were converted to enzyme activity using a standard calibration curve of 3-o-methylfluorescein (Sigma Chemicals). The fluorescence was measured using a Turner fluorometer No. 110, equipped with Turner lamp no. 110-853, in combination with 47 B primary and 2a-12 secondary filters. Autoclaved lake water with substrate added was used as a control.

Chlorophyll a was determined spectrophotometrically following extraction with acetone and correction for pheophytin (APHA (1002-G), 1985). Total soluble P, TSN, NH_4-N, and SRP were determined by standard methods (APHA 1985).

Sediments. Alkaline phosphatase activity was determined by a method adapted from Sayler et al (1979). Sediment samples (1 g wet sediment) were placed in centrifuge tubes. Three mL of 1 M Tris-Tris HCl buffer (pH 7.0) were added to each tube. The samples were sonicated (Heatsystems Ultrasonics Model W-220F) for 45 sec at 30% relative output, to release cell bound phosphatase. The samples were then incubated at 25°C, with 1 mL p nitrophenyl phosphate, at a concentration of 50 mg mL^{-1} (Sigma Chemicals) for 1 hr. At the end of the incubation 3 mL of 1 M NaOH were added to the tubes to stop the reaction and enhance p-nitrophenol color. To account for color interference controls were obtained by incubating sediment without the substrate and subsequently adding the substrate along with NaOH at the end of the incubation. All samples were then centrifuged at 7000 rpm (7996 g) for

16 min. The liquid was removed and absorbance at 410 nm (Shimadzu Model UV-160) measured. Concentrations were determined by calibration with a standard curve of *p*-nitrophenol (Sigma Chemicals).

Porewater was extracted by centrifuging a subsample of sediment at 6000 rpm (5213 g) for 15 min. The porewater was immediately filtered through 0.45 μm Gelman membrane filters. Total P, TSP and SRP were measured using standard methods (APHA 1985). Porewater total organic P (TOP) was defined as the difference between porewater TP and SRP. Bicarbonate extractable P was determined after porewater extraction by shaking the sediment with 0.6 M $NaHCO_3$ (1:50, on a dry weight basis) for 30 min. The extracted medium was then centrifuged at 6000 rpm (5213 g) for 15 min and the supernatant filtered through Whatman 40 filter paper. Total P and SRP were determined on the filtrate (APHA 1985). Labile inorganic P was calculated as the sum of porewater SRP and HCO_3 extractable SRP. Labile organic P was defined as the difference between total labile P and labile inorganic P.

Water content of the sediment was determined by drying a known weight of wet sediment at 70°C to a constant weight. The dried sediment was ground to a powder using a Spex 8000 grinding mill. Total P content of the dried sediment was determined via ignition (Walker and Adams 1958). Volatile solids were reported as the loss in weight due to ignition of dried sediment at 500°C for 2 h.

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS 1985), Version 6.03 for personal computers. Data in the first experiment were analyzed using repeated measures analysis which accounts for the inherent within replicate correlation due to repeated sampling of the same flasks. When data were unbalanced repeated measures analysis could not be used, a split plot design, with time as the subplot, was used to produce the analysis of variance and F test. Data in both experiments were analyzed using Pearson correlation coefficients.

Results

The Effect of Dissolved Oxygen on Organic Phosphorus Mineralization in the Water Column

Chlorophyll a concentrations of the water samples collected in July 1990 were extremely high (Table 5-1) and exceeded previous measurements (chapter 2). Other parameters measured were within the same range as previously recorded in Lake Apopka (chapter 2).

The response of the measured parameters over time was highly varied under both aerobic and anaerobic conditions. During the 24 h experimental period pH decreased from 8.2 to 7.5 and 8.0 under aerobic and anaerobic conditions, respectively (data not shown). Total soluble N exhibited significant differences over time ($p=0.02$). Under aerobic conditions TSN increased within 2 h and subsequently declined over time (Fig. 5-3a). Under anaerobic conditions the only significant difference observed was the increase at 24 h versus initial concentrations. There was an apparent decrease in NH_4-N concentrations over time (Fig. 5-3b), however, no statistically significant time or treatment effects were determined for NH_4-N. Both TSP and SRP concentrations also exhibited

Table 5-1. Concentrations of selected parameters measured in Lake Apopka water in July 1990 (mean ± 1 SE).

Parameter	Concentration
Chlorophyll *a* ($\mu g\ L^{-1}$)	220 ± 4
Total APA ($nM\ min^{-1}$)	21.9 ± 0.1
Soluble APA ($nM\ min^{-1}$)	0.82 ± 0.4
TSP ($\mu g\ L^{-1}$)	15 ± 0
SRP ($\mu g\ L^{-1}$)	1 ± 0
TSN ($mg\ L^{-1}$)	2.48 ± 0.0
NH_4-N ($mg\ L^{-1}$)	0.05 ± 0

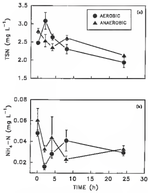

Fig 5-3. Nutrient concentrations in Lake Apopka water incubated in the dark under aerobic and anaerobic conditions. a) total soluble nitrogen, b) NH$_4$ N Vertical bars represent 1 SE. Absence of bar indicates SE is smaller than the symbol size.

insignificant time and treatment responses (Fig. 5-4a and b). SRP concentrations were below detection (< 1 $\mu g\ L^{-1}$) for both treatments after 24 h. A significant treatment response was observed for total APA (Fig 5-5a). Under anoxic conditions total APA remained constant over the 24 h sampling period. Under aerobic conditions total APA increased within 2 h and then remained constant until 24 h. At 24 h, the total APA had increased from 27 to 43 nM min^{-1}. Soluble APA accounted for 4% of total APA (Table 5-1). No change in soluble APA was observed in either treatment (Fig. 5-5b).

Significant correlations among the water chemistry parameters measured were observed. A significant inverse relationship between NH_4-N and TSP was determined (r=-0.79), and significant positive correlations between SRP and TSN (r=0.67) and NH_4-N (r=0.70) were observed.

The Effect of Redox Potential on Organic Phosphorus Mineralization in the Sediment

The initial pH of the sediment prior to the month long incubation was 7.12. Following equilibration at different Eh levels slight changes in pH values were recorded (Table 5-2). Redox values were expressed as pE + pH to account for the variability in pH among the reaction vessels (pH range 6.35 to 7.15). pE was calculated as,

$$pE = Eh / 59$$

A significant negative relationship was observed between APA and pE + pH (Fig. 5-6a). Conversely, a significant positive relationship between pE + pH and porewater TOP was recorded (Fig. 5-6b). pE + pH accounted for 77% of the variability in APA and 73% of porewater TOP

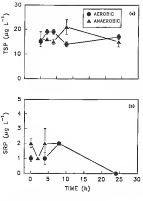

Fig 5-4. Nutrient concentrations in Lake Apopka water incubated in the dark under aerobic and anaerobic conditions: a) total soluble phosphorus, b) soluble reactive phosphorus. Vertical bars represent 1 SE. No vertical bar indicates SE is smaller than the symbol size.

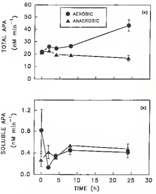

Fig. 5-5. Alkaline phosphatase activity in Lake Apopka water incubated in the dark under aerobic and anaerobic conditions. a) total alkaline phosphatase activity, b) soluble alkaline phosphatase activity. Vertical bars represent 1 SE. No vertical bar indicates SE is smaller than the symbol size.

Table 5-2. Concentrations of selected parameters measured on sediments incubated under six different redox levels for one month (mean ± 1 SE)

Eh	pH	pE + pH	APA	Porewater SRP	Porewater TOP	Labile P_i	Labile P_o
mV			μmol g dry wt^{-1} h^{-1}	mg P kg dry wt^{-1}			
483	6.35	16	6.89 ± 0.21	26 ± 0.45	2.1 ± 0.45	48 ± 1	11 ± 3
338	6.49	12	4.01 ± 0.65	51 ± 0.78	7.0 ± 2.81	74 ± 3	16 ± 3
48	6.78	8	3.64 ± 0.39	8 ± 1.19	13.8 ± 2.51	23 ± 2	19 ± 2
-2	6.55	7	4.23 ± 0.31	58 ± 0.90	8.3 ± 3.15	75 ± 0	10 ± 2
-157	6.78	4	1.07 ± 0.59	44 ± 1.35	16.1 ± 1.98	66 ± 2	22 ± 2
-242	7.15	3	0.71 ± 0.24	111 ± 1.80	18.3 ± 0.55	144 ± 2	19 ± 1

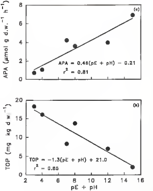

Fig. 5-6 Concentration of selected parameters measured in sediments incubated under six different redox levels for one month: a) alkaline phosphatase activity, b) porewater total organic phosphorus

variability. Non linear redox effects were observed for other P compounds. A significant increase in porewater SRP and labile inorganic P were observed at a pE + pH value of 3 (Table 5-2). In contrast FA-TP decreased at a pE + pH of 3. The HA-TP fraction tended to increase under pE + pH values of 7. However, the majority of P forms measured were not significantly affected by redox potential.

Volatile solids were 66% in both aerobic and anaerobic treatments. The sediment TP as determined by ignition was 1047 µg g dry wt.$^{-1}$. On average the total recoverable P determined by the summation of HCl-TP, NaOH-TP and porewater TP accounted for 74% of TP as determined by ignition (Table 5-3). Acid hydrolyzable-TP extracted the largest percentage of P (41%), followed by alkali extractable P (28%). Labile inorganic and organic P were a small component of the TP, contributing 5.8 (2-14) and 1.4 (1-2)%, respectively. Readily available, i.e. porewater SRP and TP, also contributed less than 20% of total P. Porewater TP accounted for 5.5 (2-13)% of the total P pool.

Total and inorganic P extracted with HCl were the same, indicating that no organic P was extracted. Porewater TSP and SRP were also equivalent suggesting that no porewater organic P was in the soluble phase; TOP represented particulate organic P.

Significant correlations between the parameters measured were observed (Table 5-4). Alkaline phosphatase activity had a high positive correlation with pE + pH ($r=0.90$) while porewater TOP was strongly inversely related to pE + pH ($r=-0.92$). Alkaline phosphatase activity was also highly negatively correlated with labile organic P ($r=-0.81$) and HA-TP ($r=-0.74$), while porewater TOP was highly positively related

Table 5-3. Concentrations of selected parameters measured on sediments incubated under six different redox levels for one month (mean ± 1 SE).

Eh	pH	pE + pH	HCl-TP	NaOH-TP FA-TP	 HA-P
mV			mg P kg dry wt.$^{-1}$		
483	6.35	15	490 ± 13	171 ± 5	132 ± 3
338	6.49	12	393 ± 26	162 ± 4	134 ± 1
48	6.76	6	431 ± 4	180 ± 1	134 ± 0
-2	6.55	7	414 ± 20	146 ± 6	126 ± 3
-157	6.76	4	421 ± 15	150 ± 15	167 ± 2
-242	7.15	3	434 ± 3	115 ± 2	148 ± 3

Table 5-4 Selected correlation coefficients between phosphorus compounds and alkaline phosphatase activity measured on sediments incubated for one month under six different redox levels

	pE + pH	APA	$LabP_i$	$LabP_o$	PW-SRP	PW-TP	PW-TOP	FA-TP	$FA-P_o$	NaOH-TP
APA	0.90*									
$LabP_i$	NS	NS								
$LabP_o$	NS	-0.81	NS							
PW-SRP	NS	NS	1.00	NS						
PW-TP	NS	NS	0.99	NS	0.99					
PW-TOP	-0.92	-0.95	NS	0.84	NS	NS				
FA-TP	NS	NS	-0.96	NS	-0.97	-0.99	NS			
$FA-P_o$	NS	NS	-0.92	NS	-0.94	-0.95	NS	0.98		
NaOH-TP	NS	NS	0.94	NS	-0.96	-0.94	NS	0.95	0.97	
$HA-P_o$	NS	-0.74[+]	NS	0.91	NS	NS	NS	NS	NS	NS

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* Unless otherwise indicated all correlations are significant at $\alpha=0.05$

[+] Significant at $\alpha=0.09$

to labile organic P (Table 5.4). A strong inverse relationship between APA and porewater TOP was observed (r=-0.95).

Other significant inverse correlations include porewater TP and SRP with FA-P and NaOH-TP. Labile inorganic P was also highly inversely correlated to these P forms. No relationship was observed between HCl-TP and any other parameters measured.

Discussion

The Effect of Dissolved Oxygen on Organic Phosphorus Mineralization in the Water Column

Phosphorus concentrations in lake water incubated in the dark for 24 h, did not show significant changes under either anaerobic (<0.2 mg L^{-1}) or aerobic (6 mg L^{-1}) conditions. However, decreases in TSN concentrations suggested that mineralization of organic N occurred, but no increase in NH_4-N was observed. This may be due to rapid nitrification under aerobic conditions (Reddy and Graetz 1981) or loss through volatilization (Stratton 1968, 1969). Initial TSN:TSP exceeded 200:1 and would suggest that the system may be P limited.

Under conditions of inorganic P limitation, phytoplankton may produce APA which hydrolyzes organic P compounds with the release of inorganic P (Kuenzler 1965). Specific APA (APA/chlorophyll *a*) ratios were determined to be an effective means of determining inorganic P limitation of Lake Apopka plankton (chapter 3). Under SRP limiting conditions, APA and specific APA increase (Kuenzler and Perras 1965; Heath and Cooke 1975; chapter 3). Initial and final specific APA ratios under aerobic conditions were 0.1 and 0.2 nmol APA µg chlorophyll a^{-1}

min^{-1}, suggesting that the plankton population was not P limited (Pettersson 1990, chapter 3). However, total APA increased from 22 to 43 nM min^{-1}. This increase could be a demonstration of either increased bacterial reproduction or alternatively, a response to decreased internal P supply within the plankton (Fitzgerald and Nelson 1966; Pettersson 1990; chapter 3). Internal P reserves, which include small chain polyphosphates (Elgavish and Elgavish 1980) are broken down in response to P demand (Rhee 1972, 1973, 1974, chapter 3). Once internal P concentrations reach a certain critical level, APA is produced (Taft et al. 1977; Sproule and Kalff 1979; Chróst and Overbeck 1987).

Under natural conditions and in continuous culture, P concentrations may be low but they are continuously replenished. In batch culture, a one time P input can result in rapid depletion of SRP, hence internal P sources may be required for metabolism (Rhee 1972). Once this P pool has been reduced to a critical level APA is produced (Chróst and Overbeck 1987). This would explain the 24 h lag time observed prior to the increase in APA observed under aerobic conditions. In contrast, under anaerobic conditions, metabolism of aerobes is reduced and eventually inhibited completely without the return of DO.

The dominant form of phytoplankton in Lake Apopka are the blue-greens, *Lyngbya* sp. and *Microcystis* sp. (Shannon and Brezonik 1972, Stites, D. L., unpublished data, St. John's River Water Management District, Palatka, FL). Under anaerobic conditions, only anaerobes and facultative anaerobes are active, however, research has demonstrated that after acclimatization cyanobacteria grow well under anaerobic conditions with H_2S acting as an electron donor in the photolysis of water during photosynthesis (Stewart and Pearson 1970). The production of APA is dependent upon the P stress within system (Fuhs et al. 1972; Kuenzler and Perras 1965). Because of the low P requirements of anaerobic bacteria, APA inhibition may occur at lower SRP levels than under aerobic conditions, where P requirements of aerobic bacteria are high. Enzyme activity did not cease under anaerobic conditions suggesting that the enzyme itself was not inhibited by anaerobic conditions. Although total APA under aerobic conditions differed from total APA under anaerobic conditions within 2 h, no significant increase in APA occurred until 24 h, this suggests that less severe low DO will not affect APA organic P mineralization. Depth profiles of APA with varying DO concentrations were shown to correlate with chlorophyll *a* and bacterial counts rather than DO concentrations (Jones 1972a), consequently metalimnetic O_2 depletion is unlikely to affect APA. However, in waters which become stratified the anoxic conditions at the sediment water interface may result in decreased APA after an extended time period. This may be due to decreased production by anaerobes or as a response to competitive inhibition by SRP released from sediments (Pettersson 1990). Lake Apopka is a frequently mixed system under which extended periods of O_2 depletion are not likely to occur. Diel changes in O_2 will be too brief (< 5 h) to affect organic P mineralization.

No change in soluble APA was observed under either aerobic or anaerobic conditions. Soluble APA has been shown to be a result of cell lysis (Berman 1970) as well as excretions by zooplankton, bacteria and phytoplankton (Wynne 1981, Pettersson 1990). It is thought that significant cell death and lysis did not occur during the period of anaerobiosis because increased nutrient concentrations and soluble APA were not observed.

The Effect of Redox Potential on Organic Phosphorus Mineralization in the Sediment

A significant effect of increasing anaerobiosis is the change from a predominately aerobic microbial population to a smaller anaerobic population. Under anaerobic conditions organic material accumulates due to reduced mineralization rates. Microbial biomass is highly correlated with APA (Sayler et al. 1979; Ayyakannu and Chandramohan 1971), and APA was significantly inhibited by the decrease in redox potential. Hence organic P mineralization will decrease under reduced conditions. In general, redox potential did not have a significant effect on the various organic P pool sizes. However, Lake Apopka sediments are poorly poised (poorly redox buffered) and the low concentrations of electron acceptors may reduce the rate of mineralization. Only APA and porewater TOP were significantly correlated with pE + pH. More resistant P forms may require longer incubation times to show a response to redox potential.

Significantly lower porewater SRP and labile inorganic P concentrations were observed at Eh 49 mV, than either Eh 339 or -2 mV. This may be a problem associated with the control of redox conditions. Redox levels may need to be established by utilizing specific electron acceptors, rather than fluctuating the air input.

Redox potential tends to decrease with sediment depth (Kobori and Taga 1979b; DeGobbis et al. 1994), while porewater SRP has often been shown to increase as a function of depth (Mortimer 1941; chapter 4).

Soluble reactive P concentrations measured at -242 mV were similar to SRP concentrations measured in the 20-40 cm depth of cores collected in January 1990 from the same site in Lake Apopka (chapter 4) indicating that porewater SRP increased in response to reduced redox potential. In Lake Apopka P fluxes were determined to be mainly a function of decomposition at the sediment-water interface (Moore et al 1991).

Aerobic conditions may also result in SRP release. Sediments may be aerated through wind induced resuspension or by diffusion of O_2 from the hypolimnion. Aerobic release of P from sediments into the overlying water is a function of the mineralization of organic P compounds (Lee et al. 1977). Greater than 90% of organic P extracted from an upland soil was shown to be in the form of phosphomonoesters (Condron et al 1985). Thus a large portion of organic P may potentially be made available through the action of phosphatase enzymes. Alkaline phosphatase activity has been significantly correlated with SRP release in marine sediments (Degobbis et al 1986). In this study a significant inverse correlation was observed between porewater TOP and sediment APA (r=-0.95), hence high APA may result in the breakdown of porewater TOP, although this may also indicate the inhibition of APA by porewater TOP. Alkaline phosphatase activity was also highly inversely correlated with labile organic P. Porewater TOP, which is already in the soluble fraction of the sediment, may be more susceptible to enzyme hydrolysis than labile organic P and hence will be hydrolyzed first. The porewater TOP pool is subsequently replenished by the labile organic P pool of the sediment. The solubility of the substrate is the factor limiting organic P mineralization not the enzyme activity (Jackman and Black

1952). It was determined that the rate of phytate hydrolysis in solution was over 71 times as important as the phytase activity; a specific acid phosphatase in the limitation of hydrolysis (Jackman and Black 1952).

The high volatile solids content of the sediment indicated the high organic matter content. A significant portion of this organic matter was highly resistent to hydrolysis, as apparent by the 26% difference in TP determined by ignition and extraction. In this study, APA was inversely correlated with HA organic P (r=-0.74), a resistant form of P. This inverse relationship may be explained as the possible binding of alkaline phosphatase to the humic substances which may increase enzyme stability, thus resulting in some inhibition of APA (Burns 1980; Kandeler 1990; Wetzel 1991). Organic P compounds may also form complexes with humates (Stewart and Tiessen 1987, Brannon and Sommers 1985). The two main chemical extractants, HCl and NaOH, were unable to extract organic P compounds incorporated into humic polymers (Brannon and Sommers 1985). This chemically resistant organic P was also resistant to enzymatic hydrolysis.

The high negative correlation between labile inorganic P and SRP with FA-TP and NaOH-TP suggest that FA-TP, a component of NaOH-TP, may be a significant contributor to the labile inorganic P pool through enzymatic hydrolysis. It has been suggested that inositol phosphates bound to FA are hydrolyzable by phytase (Herbes et al. 1975)

Conclusions

The results from this study show that short-term DO depletion will not affect APA in the water column of Lake Apopka. However, extended periods of anoxia (8 to 24 h) will result in APA inhibition and decreased enzymatic breakdown of organic P. In the sediment, APA was high under aerobic conditions and decreased with a decrease in Eh, hence under anaerobic conditions the rate of organic P mineralization will be slower. Inverse correlations between APA and porewater TOP and labile organic P also suggest that these are susceptible to enzymatic hydrolysis or may be inhibited by high concentrations of these substrates. Based upon the resistant nature of HA-TP the inverse relationship between HA and APA was attributed to the binding of the enzyme in the formation of humic complexes which accumulate under anaerobic conditions.

CHAPTER 6
ORGANIC PHOSPHORUS CYCLING IN LAKE APOPKA

Organic P plays a dominant role in the P cycle of aquatic systems (Fig. 1-1). The bioavailability of organic P to plankton is regulated by the activity of enzymes, types of substrates and associated physico-chemical factors in the system. The research presented in this dissertation examined the specific organic P compartments (plankton, water column and sediment) to evaluate the bioavailability of organic P for plankton growth. Results obtained in this study are summarized in the context of addressing key research issues raised in an attempt to understand the P dynamics in Lake Apopka.

(1) How is the enzymatic hydrolysis of organic P affected by other water chemistry parameters?

Both seasonal and spatial variability of total P (TP), total soluble P (TSP) and alkaline phosphatase activity (APA), were observed in the water column. Alkaline phosphatase activity, an indicator of potential organic P hydrolysis, was dependent upon different water chemistry parameters, both seasonally and spatially. The majority of APA was associated with particulate matter, and soluble APA averaged only 3% of total APA, therefore particulate interactions are a key component of organic P cycling in Lake Apopka. The attachment of enzymes to surfaces may increase enzyme longevity, but may also reduce enzyme activity, either directly through sorption at the active site or indirectly as a result of steric hindrance. Evidence of this was observed by the inverse relationship between APA and total organic C (TOC).

Alkaline phosphatase activity was higher in the pelagic zone (sites 2-8), which was more productive and nutrient rich than the spring (site 1). The mean annual total APA in the water column was 19 nM min^{-1}, under non-limiting substrate concentrations, this indicates a potential inorganic P release rate of 0.6 μg P L^{-1} min^{-1}. Natural substrate concentrations are usually lower than those used in the APA assay, thus *in situ* release rates will be slower.

(2) Is Lake Apopka plankton APA inhibited by inorganic P and is it produced in response to inorganic P limitation?

Over 90% of APA within the natural plankton population was inhibited following the addition of 1000 μg L^{-1} inorganic P to the growth medium. Addition of lower inorganic P concentrations (10 to 100 μg L^{-1}) did not produce such complete inhibition, thus ambient soluble reactive P (SRP) concentrations (< 10 μg L^{-1}) in the lake water will not inhibit APA.

The uptake of added inorganic P was very rapid, indicating high P demand. The inorganic P was immediately incorporated into the plankton cells as surplus P, as determined by hot water extraction. In general, surplus P was inversely related to APA, suggesting that internal inorganic P levels controlled APA. Under field conditions this was reflected by an inverse relationship between acid hydrolyzable P and APA. In batch culture, both hot water extractable inorganic and organic P were used to provide P for growth.

Increased APA was associated with increased plankton biomass, however, the intensity of APA was dependent upon the severity of P limitation. During severe inorganic P limitation Lake Apopka plankton produced specific APA (total APA/chlorophyll a) values > 1 nmol APA μg chlorophyll a^{-1} min^{-1}. Ambient lake water specific APA was < 0.3 nmol APA μg chlorophyll a^{-1} min^{-1}, suggesting that Lake Apopka plankton were not severely P limited.

(3) What effect does sediment resuspension have upon organic P mineralization rates?

Resuspension of surficial sediments resulted in immediate increases in total suspended solids (TSS), total Kjeldahl N (TKN), TP and APA within the overlying water column. These elevated concentrations decreased rapidly upon particle settling, indicating they remained associated with the sediment particles. During resuspension, increased APA and TP concentrations within the overlying water column may result in increased organic P mineralization. Exposure of anaerobic sediments to the oxygenated water column during resuspension may also increase the rate of organic P mineralization within the sediment itself. Porewater organic P was inversely related to pE + pH, while APA was positively related to pE + pH, indicating reduced mineralization rates of organic P under anaerobic conditions. Alkaline phosphatase enzymes have been shown to bind to humic acid complexes, the inverse relationship between humic acid P (HA-TP) and APA indicates inhibition in response to binding. The overall conclusion is that mineralization rates may be increased as a result of sediment resuspension and aeration, and are reduced under anaerobic conditions.

The results presented in this study demonstrate significant potential for organic P mineralization in Lake Apopka. Substrates hydrolyzable by APA are very labile, hence the rate of organic P mineralization in Lake Apopka is limited by substrate concentrations rather than enzyme activity. Further research should focus more on specific organic P compounds within the sediment water column and their relative turnover rates. Studies should also include evaluating the change in enzyme kinetics in response to different organic P compounds. To encourage P limitation, emphasis should be placed upon a means to inhibit organic P mineralization.

APPENDIX A
LORAN COORDINATES

Table A. Loran coordinates of sampling sites on Lake Apopka Group repetition interval 7980, Southeast USA.

Site number	Time delay µs Y	Z
1	44526.6	62460.6
2	44524.2	62446.4
3	44553.9	62452.3
4	44577.2	62449.9
5	44539.7	62416.6
6	44497.1	62403.0
7	44488.6	62420.2
8	44530.0	62432.2

APPENDIX B
CONCENTRATIONS OF SELECTED WATER CHEMISTRY PARAMETERS DETERMINED BIMONTHLY FROM APRIL 1989 THROUGH FEBRUARY 1990, AT 8 SITES IN LAKE APOPKA

Table B-1. Temperature.

Date	Site 1	2	3	4	5	6	7	8	Mean*	SE
					°C					
APR	24.3	27.4	27.4	28.7	26.3	28.1	28.1	23.6	26.6	0.21
JUN	24.2	32.5	30.4	28.5	30.0	29.8	28.1	29.8	29.9	0.20
AUG	24.0	28.7	28.0	27.8	29.5	29.8	29.2	28.5	28.8	0.11
OCT	22.7	21.1	19.9	19.8	19.4	19.9	20.0	19.4	19.9	0.08
DEC	20.5	17.6	18.5	15.8	16.3	15.7	15.0	15.5	16.1	0.12
FEB	22.3	20.1	19.8	20.6	20.8	20.4	21.3	20.0	20.0	0.07
Mean	23.1	25.5	24.4	23.7	24.3	24.3	24.1	23.4		
SE	0.3	1.0	1.0	0.9	1.0	1.0	1.0	1.0		

* Means listed in this column are means of sites 2-8.

Table 8.2. Secchi depth transparency

Date	Site								Mean*	SE
	1	2	3	4	5	6	7	8		
	m									
APR	1.00	0.24	0.20	0.25	0.23	0.24	0.23	0.25	0.23	0.00
JUN	1.50	0.21	0.21	0.24	0.24	0.21	0.24	0.21	0.22	0.00
AUG	1.90	0.20	0.23	0.30	0.25	0.25	0.23	0.23	0.24	0.00
OCT	1.38	0.27	0.28	0.28	0.27	0.35	0.24	0.30	0.28	0.00
DEC	1.00	0.25	0.25	0.39	0.31	0.32	0.25	0.27	0.29	0.01
FEB	0.72	0.28	0.24	0.30	0.25	0.22	0.25	0.25	0.25	0.00
Mean	1.36	0.23	0.23	0.29	0.28	0.27	0.24	0.25		
SE	0.06	0.00	0.01	0.01	0.01	0.01	0.00	0.01		

* Means listed in this column are means of sites 2-8.

Table B-3. Dissolved oxygen.

Date	Site 1	2	3	4	5	6	7	8	Mean*	SE
	$mg\ L^{-1}$									
APR	4.5	12.7	13.0	11.2	12.3	12.8	13.0	10.7	12.2	0.13
JUN	2.4	7.3	10.5	3.7	5.2	8.8	6.3	9.8	7.3	0.35
AUG	2.5	8.7	9.3	5.8	10.2	11.0	10.8	9.6	9.3	0.25
OCT	4.2	9.7	8.6	8.9	9.1	9.6	10.6	10.2	9.5	0.10
DEC	5.3	9.5	9.0	9.9	9.1	9.9	10.1	9.3	9.5	0.06
FEB	5.5	7.7	10.2	10.0	9.6	10.8	12.1	9.0	9.9	0.20
Mean	3.8	9.6	10.1	7.9	9.2	10.3	10.2	9.9		
SE	0.2	0.3	0.3	0.5	0.4	0.3	0.4	0.1		

* Means listed in this column are means of sites 2-8

Table 8-4. pH.

Date	Site								Mean'	SE
	1	2	3	4	5	6	7	8		
APR	8.2	9.4	9.3	9.1	9.3	9.4	9.4	9.2	9.3*	0.0
JUN	8.1	8.8	9.0	8.1	8.8	9.0	8.5	9.1	8.8	0.1
AUG	8.1	8.9	9.1	7.8	8.9	9.2	9.3	8.9	8.5	0.1
OCT	8.2	8.7	8.9	8.7	9.0	9.2	9.1	9.0	8.9	0.0
DEC	8.2	9.0	8.8	9.1	8.9	8.9	9.0	9.0	8.9	0.0
FEB	8.1	8.5	8.7	8.7	8.2	8.5	8.8	8.5	8.5	0.0
Mean	8.2	8.8	8.9	8.3	8.7	8.9	8.9	8.9		
SE	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0		

' Means listed in this column are means of sites 2-8

* Means were calculated following the conversion of pH to hydrogen ion concentrations. Standard errors were calculated without prior conversion of pH values

Table B-5 Total solids

Date	Site								Mean[a]	SE
	1	2	3	4	5	6	7	8		
					mg L^{-1}					
APR	ND[b]	ND	ND	ND	ND	ND	ND	ND		
JUNE	148	349	373	337	411	411	300	404	369	6
AUG	156	309	317	328	334	335	323	350	328	2
OCT	202	387	396	385	402	395	402	411	397	1
DEC	229	369	369	389	370	373	393	396	380	2
FEB	271	531	499	524	511	459	496	508	504	3
Mean	201	389	391	393	406	395	383	414		
SE	10	17	13	16	13	9	15	12		

[a] Means listed in this column are means of sites 2-8

[b] ND indicates not determined

Table B-6 Total suspended solids

Date	Site								Mean*	SE
	1	2	3	4	5	6	7	8		
	$mg\ L^{-1}$									
APR	ND•	ND	ND	ND	ND	ND	ND	ND		
JUN	ND	ND	ND	ND	ND	ND	ND	ND		
AUG	6	59	66	42	49	69	72	73	62	2
OCT	5	54	67	52	71	60	64	75	63	1
DEC	15	61	69	48	67	60	58	65	61	1
FEB	17	77	85	108	99	89	92	103	94	2
Mean SE	11 2	63 2	72 2	63 8	72 5	70 3	72 4	79 4		

* Means listed in this column are means of sites 2-8

• ND indicates not determined

Table 8-7 Chlorophyll a

Date	Site								Mean*	SE
	1	2	3	4	5	6	7	8		
	μg L⁻¹									
APR	33	61	70	68	76	71	52	98	71	2
JUN	24	80	79	98	84	92	55	67	78	2
AUG	24	131	142	87	156	95	158	127	128	4
OCT	16	92	96	75	101	73	85	99	99	2
DEC	26	102	94	60	68	71	79	74	78	2
FEB	7	39	33	49	48	51	40	45	44	1
Mean	22	84	86	71	89	75	78	85		
SE	1	5	6	3	6	3	7	5		

* Means listed in this column are means of sites 2-8

Table 8-8 Total organic carbon.

Date	Site								Mean*	SE
	1	2	3	4	5	6	7	8		
	mg L^{-1}									
APR	ND•	ND	ND	ND	ND	ND	ND	ND		
JUN	0.3	30.8	29.9	29.8	31.6	31.5	24.3	32.2	30.0	0.38
AUG	2.4	32.5	34.0	40.5	32.4	31.7	33.1	34.0	34.0	0.43
OCT	1.2	28.5	29.8	30.5	29.0	32.9	28.3	29.2	29.7	0.22
DEC	9.1	30.1	28.6	29.4	31.1	32.9	27.8	31.9	30.3	0.26
FEB	10.6	35.2	36.9	33.7	44.5	32.6	34.0	39.2	36.6	0.59
Mean	4.7	31.4	31.8	32.8	33.7	32.3	29.5	33.3		
SE	0.95	0.51	0.69	0.93	1.23	0.13	0.81	0.75		

* Means listed in this column are means of sites 2-8

• ND indicates not determined

Table B-8. Total Kjeldahl nitrogen.

Date	Site								Mean*	SE
	1	2	3	4	5	6	7	8		
	mg L^{-1}									
APR	2.57	5.06	6.12	5.38	5.71	5.03	5.18	8.11	5.80	0.16
JUN	0.18	4.71	4.72	4.62	4.78	5.33	3.58	4.48	4.60	0.07
AUG	1.30	5.28	6.45	4.86	5.56	5.53	5.66	5.25	5.51	0.07
OCT	0.61	2.08	3.82	3.64	4.68	3.14	4.08	4.28	3.67	0.12
DEC	2.30	4.19	4.72	3.81	4.13	4.36	4.59	4.45	4.32	0.04
FEB	1.87	4.04	4.12	4.85	5.30	4.58	5.06	4.00	4.56	0.08
Mean	1.47	4.23	4.99	4.53	5.03	4.66	4.68	5.09		
SE	0.16	0.18	0.18	0.11	0.10	0.14	0.13	0.26		

* Means listed in this column are means of sites 2-8.

Table 8-10 Total and soluble alkaline phosphatase activity.

Date	Site								Mean*	SE
	1	2	3	4	5	6	7	8		
	nM min^{-1}									
Total										
APR	5.9	12.2	12.8	22.0	18.7	20.1	20.7	22.8	18.5	0.61
JUN	3.8	11.8	11.2	44.7	29.9	30.6	20.8	19.2	24.0	1.70
AUG	3.7	10.2	10.6	10.3	6.4	17.8	20.8	11.6	12.5	0.71
OCT	5.7	27.0	28.2	19.2	19.6	21.9	25.4	24.9	23.6	0.54
DEC	7.7	23.8	24.6	19.8	18.8	17.9	20.3	17.4	20.4	0.40
FEB	5.6	9.6	10.3	11.6	9.5	10.6	10.9	8.9	10.2	0.13
Mean	5.4	15.8	16.3	21.1	17.2	19.8	19.8	17.5		
SE	0.25	1.26	1.33	2.07	1.39	1.08	0.80	1.04		
Soluble										
APR	0.16	0.51	0.49	0.34	0.29	0.67	0.37	0.30	0.42	0.02
JUN	0.07	0.03	0.05	0.93	0.01	0.07	0.10	0.20	0.20	0.05
AUG	0.31	0.10	0.54	0.60	0.35	0.16	0.55	0.64	0.42	0.03
OCT	0.74	1.29	1.19	0.64	0.48	0.80	0.29	0.27	0.71	0.06
DEC	0.65	0.38	0.62	0.45	1.02	0.69	0.58	1.34	0.72	0.05
FEB	0.39	0.56	0.33	0.43	0.11	0.12	0.03	0.09	0.24	0.03
Mean	0.39	0.48	0.53	0.57	0.39	0.42	0.32	0.47		
SE	0.04	0.08	0.06	0.04	0.06	0.06	0.04	0.08		

* Means listed in this column are means of sites 2-8.

Table 9-11. Phosphorus.

Date	1	2	3	4	5	6	7	8	Mean*	SE
					µg L⁻¹					
TP										
APR	110	140	170	180	200	200	180	260	190	5
JUN	30	150	140	190	150	170	260	140	171	6
AUG	30	140	90	240	240	130	120	230	170	9
OCT	300	520	440	350	200	400	270	400	369	15
DEC	20	100	120	110	130	120	150	150	126	3
FEB	70	190	200	250	280	230	240	240	233	4
Mean	93	207	193	220	200	208	203	237		
SE	10	26	21	13	9	17	10	16		
TSP										
APR	130	110	70	70	90	80	50	40	73	3
JUN	80	40	70	70	80	60	30	40	56	3
AUG	10	10	10	10	10	10	10	10	10	0
OCT	120	170	70	130	60	140	160	60	113	7
DEC	10	10	10	20	20	10	10	20	14	1
FEB	30	40	40	40	40	20	20	20	31	2
Mean	63	63	45	57	50	53	47	32		
SE	9	11	5	7	5	9	10	3		
SRP										
APR	1	3	3	5	3	5	2	3	3	0
JUN	2	3	4	4	5	3	3	5	4	0
AUG	4	7	5	4	6	4	4	5	5	0
OCT	1	1	1	1	1	1	1	1	1	0
DEC	1	1	1	1	1	1	1	1	1	0
FEB	0	3	0	1	1	1	2	2	1	0
Mean	2	3	2	3	3	3	2	3		
SE	0	0	0	0	0	0	0	0		

* Means listed in this column are means of sites 2-8.

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BIOGRAPHICAL SKETCH

Susan Newman was born 26 June 1963 in Portsmouth, England. She graduated with a BSc. Honors degree in management and chemical sciences, from the University of Manchester Institute of Science and Technology, in June 1984. Following a 6 month appointment at the Weed Research Organization (the now defunct WRO), Susan determined it was time to leave the cloudy skies of England. With emotions split between excitement and trepidation, Susan began a Master of Science degree in the Agronomy Department at the University of Florida in January 1985. With an interest in wetland soils dating back to the making of mud pies during her childhood, and unable to resist the opportunities of the Sunshine State, she began a Ph.D. program in the Soil Science Department at the University of Florida in 1987. The first course she completed within this department was openwater scuba diving, however, following that the more traditional courses were taken. Upon completion of her Ph.D. Susan will continue her aquatic/wetland science interests by obtaining a research position to investigate the nutrient dynamics in wetlands.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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